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Der Pharma Chemica, 2015, 7(1):10-13
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Analysis of nitrosodiethylamine (NDEA) in Indonesia salted fish with hollow fiber-liquid phase microextraction gas chromatography flame ionization detector

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ABSTRACT

Hollow fiber liquid phase microextraction (HF-LPME) is one of sample preparation techniques that is used for separating analyte from sample and enriching the analyte. In this research, HF-LPME-GC-FID is applied for analyzing NDEA compound in salted fish from traditional market and supermarket. This method is supported with gas chromatography completed by flame ionization detector as a detector. The optimization of analytic parameters is ethyl acetate as organic solvent, the optimum stirring rate is 252 rpm, and the optimum temperature is 30°C. The linier calibration curve of NDEA analysis with HF-LPME-GC-FID technique have coefficient correlation (R^2) 0.9998, limit of detection 0.645 ppb, accuracy between 99.61-100.16%, precision between 0.24-1.33%, and enrichment factor 999.8 times.

Keywords: Hollow fiber, liquid phase microextraction, nitrosodiethylamine.

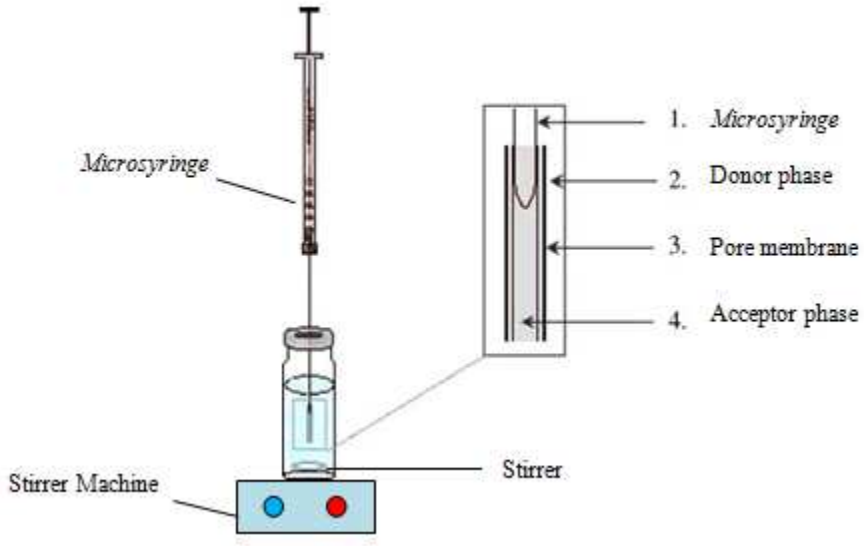
INTRODUCTION

Separation method is an important aspect in the field of chemistry because most materials found in nature in the form of a mixture of compounds. Mixture of pure material can be obtained from separation method. Extraction stage aims to isolate and concentrate the analytes from sample matrix [1]. One of the extraction techniques is hollow fiber liquid phase microextraction (HF-LPME) technique. Hollow fiber liquid phase microextraction is a microextraction method using hollow fiber as a medium of mobilization analyte. This method is the simplest method and is able to measure the concentration of analyte in a small ceoncentration (until ppb range) [2]. The principle of this method is to avoid the organic solvent use by into the hollow fiber and put in the sample solution, the solvent is drawn back into the syringe. Furthermore, a solvent that has been drawn can be analyzed by gas chromathography (GC) or high performance liquid chromathography (HPLC). HF-LPME technique is divided into two types of micro-HF-LPME extraction of the two-phase and three-phase HF-LPME [3]. In two-phase HF-LPME method, the analytes extracted from the sample solution (donor phase) through the organic solvent immobilized in the pores of the hollow fiber into the same organic solvent (acceptor phase) whereas the HF-LPME method of three-phase analytes extracted from sample solution (donor phase) through the organic solvent immobilized in the pores of the hollow fiber membrane into another aqueous phase (acceptor phase).

The use of nitrite salts in food preservation processes such as fish and meat can cause harmful effects [4]. Nitrites can bind to an amino or amide derivative to form toxic nitrosamine. In the present study, analyzes derived nitrosamine compounds that nitrosodiethylamine (NDEA) in salted fish using gas chromatography-flame ionization detector (GC-FID) technique by extraction with HF-LPME method. The parameters studied are the stirring speed, temperature and volume of sample solution.

MATERIALS AND METHODS

Chemicals used in this study include nitrosodiethylamine (NDEA) of 99.9%, organic solvent (ethyl acetate, toluene, n-hexane, and methanol) (p.a.), aquadem, hollow fiber polypropylene with a diameter of 600 µm, membrane wall thickness of 200 µm, and pore size of 0.2 µm were ordered in the membrane. The samples are dried fish obtained from supermarkets in Mulyosari, Surabay, Indonesia and traditional markets in Kenjeran, Surabaya, Indonesia. The instruments used in this study are gas chromatography-flame ionization (GC-FID) detector type Agilent 6890 plus GC version A.03.08, microsyringe, micropipette, magnetic stirrer, stir bar, vials, stirrer machine, and glassware commonly used in the laboratory.



Picture 1. *Set-up* HF-LPME

In this study the concentration of NDEA standard solution used is in the range of 50-90 ppm and optimized analytical parameters include the type of organic solvent, stirring speed, and temperature. At each optimization parameters replication is performed three times for validation and optimization of each parameter measurement is done separately. Step working outline for the optimization of each parameter is as much as 20 mL of standard solution of 70 ppm is inserted into the bottle and stir with magnetic stirrer, then covered with a rubber cover. Microsyring tip contains organic solvents 3 µL inserted into the vial bottle stopper inserted into 1.5 cm long hollow fiber. Closing vial which has been fitted with a microsyringe inserted vertically in the vial until all the hollow fiber immersed in the standard solution. Then the tip of a microsyringe pressed. Standard solution was then stirred with a magnetic stirrer. The extraction process is done for 15 minutes, after the extraction process is complete, the organic solvent was withdrawn into the microsyringe 1 mL then diluted back to 20 times and then injected directly into the GC-FID instrument, the analysis results in the form of chromatogram peak area.

RESULTS AND DISCUSSION

Preparation of NDEA standard curve without HF-LPME

In this study, standard curve of NDEA conc. without extraction are made on 50, 60, 70, 80, and 90 ppm. The concentration of the selection is based on the ability of the GC-FID minimal instrument that can detect NDEA analytes only at concentrations above 40 ppm. Calibration curve regression equation NDEA analysis without HF-LPME-GC-FID $y = 0.495x - 15.05$ with r^2 value of 0.9995.

Optimization of organic solvent type

The selection of organic solvents based on principles like disolve like, the level of selectivity, polarity, volatility, density, surface tension, boiling point, and a dielectric constant also determines the optimization of organic solvents in the extraction process of the analyte. In addition, the organic solvent should ideally be attached to the hollow fiber, insoluble in water, and does not easily evaporate during the time of extraction.

In the optimization process of the type of solvent with HF-LPME, the condition is made permanent NDEA concentration 70 ppm, temperature 30°C, stirring speed 252 rpm, extraction time of 15 minutes, the volume of organic solvent 3 mL and 20 mL volume of the sample solution. After the extraction process is complete, the

organic solvent in the hollow fiber as much as 1 mL drawn into the syringe and diluted up to 20 times in Eppendorf, then pulled back as much as 3 µL analytes syringe injected into GC.

Table 1. Organic solvent optimization results

Type of organic solvent	Average area (unit)
Toluene	48.40
n-Hexane	49.74
Ethyl acetate	217.10

This result is obtained because of solubility NDEA in ethyl acetate greater than in n-hexane and toluene. Optimum results in ethyl acetate solvent is affected by the value of log K_{ow} NDEA (0.48) which is almost equal to the value of log K_{ow} ethyl acetate (0.66) so that the NDEA solubility in ethyl acetate higher than in n-hexane and toluene. Log K_{ow} values affect the level of polarity where the smaller the value of log K_{ow}, the higher the polarity level. In addition, in terms of the value is known that ethyl acetate has a dielectric constant value greater than that of other solvents and smaller compared to methanol, so that the NDEA more soluble in ethyl acetate. The greater the value of the dielectric constant, the greater the degree of polarity.

Temperature optimization

In the optimization process temperature on HF-LPME method, which was made permanent condition is 70 ppm NDEA conc., stirring speed 252 rpm, extraction time of 15 minutes, the volume of organic solvent 3 mL, 20 mL volume of the sample solution and using ethyl acetate with a temperature variation of 25, 30, and 35°C. After the extraction process is complete, the analytes in the hollow fiber as much as 1 mL drawn into the syringe and diluted up to 20 times in Eppendorf, then pulled back as much as 3 µL analytes syringe injected into GC.

Table 2. Temperature optimization results

Temperature (°C)	Average area (unit)
25	27.25
30	217.10
35	66.76

The data obtained showed that at a temperature of 25°C analyte transfer that occurs extremely low, this is because the size of the kinetic factors that occur so that the analyte is less than optimal move from the sample solution into an organic solvent. At temperatures of 30°C occurs most optimum analyte transfer and at a temperature of 35°C decreased analyte transfer again, this is because at temperatures above 30°C will experience the desorption analyte so that the analyte has been extracted into an organic solvent transfer back into the sample solution, in addition due to desorption events, a decrease in analyte transfer also due to reduced organic solvent which can extract the analytes due to the organic solvent evaporation factor [5]. It is proved that an increase in temperature above the optimum temperature will result in the distribution of analyte present in the sample solution and organic solvents disturbed.

Stirring speed optimization

In this research, the stirring speed optimization that aims to produce maximum extract analytes. Stirring intensity is one of the important parameters on the efficiency of extraction, the stirring can increase mass transfer rates during the extraction process takes place [6]. Stirring speed affects the thermodynamic analytes during the extraction process, where with stirring, the time to reach thermodynamic equilibrium shorter, but if the speed exceeds the limit of optimal mixing will occur through the transfer process.

Table 3. Stirring speed optimization results

Stirring speed (rpm)	Average area (unit)
126	35.32
252	217.10
378	55.82

Stirring speed serves to reduce the time of attainment of the thermodynamic equilibrium of the analyte, so that when the stirring speed in the range 126 rpm, the analyte can not be absorbed by organic solvents, this is because the speed of 126 rpm extraction takes longer than 15 minutes to reach thermodynamic equilibrium where appropriate organic solvent saturated by the analyte. At the time of the stirring speed in the range 378 rpm wide analyte chromatograms generated NDEA actually getting smaller, it is due to mixing processes that exceed the limit will lead to optimum thermodynamic equilibrium has been disturbed so that the extracted analytes will experience collisions with water molecules, ethyl acetate and methanol strongly, causing the analyte to diffuse back into the

sample solution [7]. The result of this optimization is used then the next most optimum stirring speed is in the range of 252 rpm.

Preparation of NDEA standard curve extraction using HF-LPME

In the present study, the manufacture of the standard curve after extraction using conditions that have been optimized analytical parameters of the organic solvent ethyl acetate, temperature 30°C and with a stirring speed of 252 rpm sample solution. Based on the data obtained by a linear regression equation is y = 7.168x - 285.6 with y is the area of the chromatogram and x is the concentration of the standard solution NDEA. From the standard curve, r^2 value of 0.9998 is obtained, the value of 0.645 ppb detection limit, accuracy value of 99.983% precision on average by 0.733%. From these results it can be concluded that HF-LPME method for the analysis of NDEA is very good because it can lower the detection limit value with good accuracy and precision [8]. Furthermore, the regression equation of standard solutions NDEA after extraction will be used to determine the concentration of analyte in the sample NDEA and to determine the recovery (%) of the sample spiking process.

Sample analysis

Sample solution is analyzed using HF-LPME extraction method in which the analytic optimization parameters according to the results of previous measurements of the sample volume 20 mL, 1.5 cm long hollow fiber, 3 mL volume of organic solvent, the organic solvent is ethyl acetate, extraction time 15 min, stirring speed 252 rpm and at a temperature of 30°C.

From NDEA concentration measurement data in salted fish can be calculated that the salted fish from supermarkets containing NDEA was 42 ppb. This is because the preservation of salted fish from supermarkets use more nitrate preservatives than salted fish from traditional market where nitrate will react with secondary amines to form nitrosamines and derivative compounds. In salted fish from traditional markets are not generated extents chromatogram area so it can be concluded that the concentration of NDEA in salted fish from traditional market below the detection limit or nearly zero. Under Decree of Indonesian Health Minister No. 722/Menkes/Per/IX/88 on food additives, maximum limits the use of preservatives nitrate in the diet of 500 mg/kg, so it can be concluded that salted fish from traditional markets and supermarkets still feasible for consumption because it does not exceed the maximum limit of nitrate content in food.

CONCLUSION

NDEA analysis process with HF-LPME technique using gas chromatography resulted in a detection limit value of 0.645 ppb, at 99.983% accuracy, precision values between 0.236-1.33% and amounted to 999.8 times concentrated factor, so it can be concluded that the technique of HF-LPME-GC-FID very well in the analysis of NDEA compounds.

Optimization of analytical parameters on NDEA analysis with HF-LPME technique using gas chromatography is the most optimum by using ethyl acetate as the organic solvent, stirring speed 252 rpm, and temperature of 30°C. In salted fish fro m supermarket obtained 42 ppb of NDEA.

Acknowledgments

The authors are grateful to Indonesia Ministry of Education for financial support, Faculty of Science and Technology and Faculty of Pharmacy, Universitas Airlangga for the facilities.

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