CHAPTER 2

MATERIALS AND METHODS

2.1 Apparatus and instruments

2.1.1 AIRmetrics MiniVol^{TM}, Portable Air sampler consists of;

1) Pump
2) Pre-separator acid Cassette Filter Holder
3) Sealed led-acid batteries, 12 AH capacity
4) 1 amp built in charger adapter

All parts are from Airmetrics, U.S.A

2.1.2 Fiberfilms filter, 47 mm T60A20 Pallflex® filter, Pall Life Sciences, U.S.A

2.1.3 Tested engine, manufactured by Febix International Co., Ltd, consists of;

1) Yanmar TF 75-LM, Yanmar S.P. Co., Ltd., Thailand
2) Aluminium single point load cell, Vishay Tedea-Huntleigh model No. 1263, Vishay Intertechnology Inc., U.S.A
3) Tested engine controller, Febix International Co., Ltd., Thailand
4) Generator, Elec King super generator CC 7.5, N.K. L Machinery Ltd., Part.
5) Heater

2.1.4 Microbalance weight, Mettler Toledo, MX5, Switzerland

2.1.5 High performance liquid chromatography consists of;

1) FCV-12AH six-port valve (Shimadzu, Kyoto, Japan)
2) LC-10AD pump (pump 1, Shimadzu, Kyoto, Japan)
3) L-6200 pump (pump 2, Hitachi, Japan)
4) LC-10AD VP pump (pump 3, Shimadzu, Kyoto, Japan)
5) Rheodyne model 7125 injector (20µL loop)
6) DGU-14A degasser (Shimadzu, Kyoto, Japan)

2.1.6 Guard column (Cosmosil 5-MS-II; 4.6mm i.d.×10mm), (Nacalai Tesque, Kyoto, Japan)

2.1.7 Clean-up column (Cosmosil 5C18-MS-II; 4.6mm i.d.×50 mm), (Nacalai Tesque, Kyoto, Japan)

2.1.8 Concentration column (Spheri-5 RP-18; 4.6mm i.d.×30mm, Chemco, Tokyo, Japan)

2.1.9 Reduction column (NPpak-RS; 4.0mm i.d.×10mm, JASCO, Tokyo, Japan)

2.1.10 Separation column (Cosmosil 5C18-AR-II; 3.0mm i.d.×250mm, Nacalai-Tesque, Kyoto, Japan)

2.1.11 CTO-2A column oven (Shimadzu, Kyoto, Japan)

2.1.12 CTO-10AS VP column oven (Shimadzu, Kyoto, Japan)

2.1.13 Ultrasonicator SU-6TH, (Sibata, Japan)

2.1.14 Rotary evaporator consists of;

1) water bath- Thermo Fuse, (Applied Yazawa, Japan)
2) Rotary evaporation, N-1000, Eyela
3) Cooling device, ECO-Clair system of organic solvent recovery
4) Dry vacuum pump - DTC-21 Diaphragm type

2.1.15 paper filter 125 mm diameter, Advantec No.6, Toyo Roshi Kaisha, Ltd., Japan

2.1.16 polytetrafloroethylene (PTFE) membrane filter for HPLC, HLC-DISK3, 3 mm diameter and HLC-DISK13, 13 mm diameter, Hydrophobic pore size 0.45 µm, Kanto Chemical Co., Inc., Japan

2.1.17 Centrifugal filter (Ultrafree-MC, Millipore) of 0.45µm pore size

2.1.18 Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.1.19 Desiccator

2.1.20 Digital balance, Mettler AJ150, U.S.A

2.1.21 Bomb calorimeter consists of;

1) Jacket
2) 18 to 30 °C thermometer graduated to 0.01 °C
3) 2,000 mL graduated cylinder
4) Bracket
5) Bucket stirrer
6) Vibrator
7) Calorimeter bucket
8) Lead wire
9) Bomb ignition unit
10) Oxygen charger

All parts are from Parr instrument company, U.S.A

2.1.22 Hydrometer apparatus consists of;

1) Glass hydrometer
2) Hydrometer cylinder

2.1.23 Flash point tester consists of;

1) Pensky-Martens close cup apparatus
2) Thermometer: - Low Range : -7 to 110 °C, Graduation 0.5 °C
   - High Range : 90 to 370 °C, Graduation 2 °C

2.1.24 Cloud and pour point bath consists of;

1) Thermometer
2) Cork
3) Test vessel
4) Gasket
5) Seta-Lec cloud and pour point refrigerator

All parts are from Stanhope-Seta Ltd., UK

2.1.25 Setavis™ kinematic viscometer model no. 83541-3, Stanhope-Seta Ltd., UK

2.2 Chemicals

The chemicals used were listed in Table 2.1. Unless otherwise specified, all chemicals used were analytical grade or higher purity.

Table 2.1 List of chemicals

Chemical	Supplier
1-nitropyrene	Sigma–Aldrich (St. Louis, MO, USA)
Deuterated 1-NP (1-NP-$_{d9}$)	C/D/N Isotopes Inc. (Quebec, Canada)
Methanol (99.5%, HPLC grade)	Wako Pure Chemical Industries Ltd., Japan

Table 2.1 List of chemicals (Continued)

Chemical	Supplier
Ethanol (for Pesticide Residue and Polychlorinated Biphenyl Analysis, 99.5%) Benzene (100%, HPLC purity grade) Ascorbic Acid (100% purity grade)	Wako Pure Chemical Industries Ltd., Japan
Acetonitrile (99.8%, HPLC grade purity grade)	Kanto Chemical Co., Inc., Japan
Dimethyl-sulfoxide (DMSO) (HPLC purity grade)	Dojindo Molecular Technologies, Inc., Japan
Ultrapure water from the Mill – Q system	Millipore, Bedford, MA, USA
n-Heptane (99%) Acetone (99.5%)	Carlo Erba, France

2.3 Software

Statistical calculations were performed using the SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA) supported by Department of Statistic, Faculty of Science, Chiang Mai University.

2.4 Sampling method

2.4.1 Sample selection

Two community-scale biodiesel (CB) sources were used in this study. Biodiesel from used palm oil from Umong district, Lamphun Province and wasted cooking oil from Sankampaeng district, Chiang Mai Province were chosen because of reliability in supplies, production rate, material sources are permanent.

Used palm oil from Umong district, Lamphun Province supplied for municipal diesel engine (Figure 2.1) .Waste cooking oil from Sankampaeng district, Chiang Mai Province supplied for car engine and agricultural purpose such as water pump, cultivator engine tractor (Figure 2.2). Both fuels were tested directly as CBU(CB100) and CBS(CB100) and also blended with reference fuel with 1:1 ratio ((CBU(CB50) and CBS(CB50)).

Figure 2.1 Biodiesel supplied for municipal diesel engine in Umong, Lamphun Province

Figure 2.2 Biodiesel supplie for diesel engine in Sankampaeng, Chiang Mai Province

2.4.2 Tested engine preparation and operating conditions

Yanmar TF 75-LM, purchased in 2007, was used in this experiment as a representative of diesel engine. The main characteristics of tested engine are cited in Table 2.2. The diagram of sampling equipment is shown in Figure 2.3. The air sampler was installed downstream of the exhaust pipe with distance of 1.5 m from the tested engine. The tested engine was operated at room temperature and was conducted at engine speed of 1,800 rpm under full engine loads. The operation and maintenance of tested engine were followed from the operation manual [21]. The fuel was supplied to the engine by external tank of 1-L capacity, which could be drained easily. For every change of fuel, the fuel line was purged out of the residual fuel. A 20-mL glass burette was also set up in parallel to fuel tank and was used for measurement of fuel flow rate. Engine performance test including fuel consumption, engine power and torque were observed to ensure the stabilizing of engine performance. Testing of tested fuel effectiveness was performed in triplicate, and the average results are reported herein.

Table 2.2 Characteristics of tested agricultural diesel engine

Item	Specification
Engine model	Yanmar TF 75-LM
Engine type	4 strokes, water-cooled diesel
Aspiration type	Natural aspiration
Injection type	Indirect injection
Number of cylinder	1, horizontal
Bore × stroke	80 × 87 mm
Displacement	437 cc
Compression ratio	23.0 : 1
Max power	5.52 kW @ 2,200 rpm
Max torque	26.48 Nm @ 1,600 rpm
Engine speed	1,800 rpm
Engine load	100%

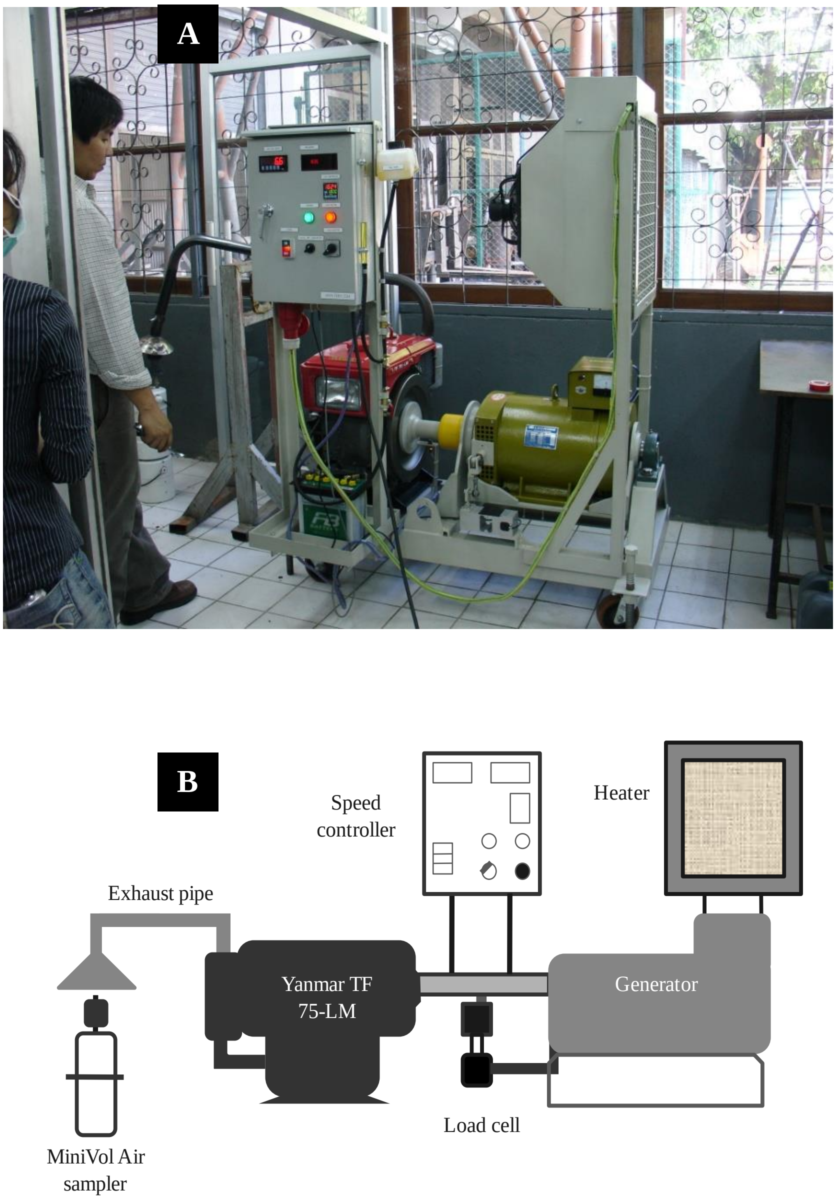

Figure 2.3 Photograph (A) and schematic (B) of tested engine

2.4.3 Sample collection

The exhaust particles from agricultural diesel engine fueled with different tested fuels were collected by MiniVol portable air sampler equipped with a glass fiber filter (with diameter of 47 mm, T60A20 Pallflex® filter). The exhaust was automatically pumped with a flow rate of 5.6 L/min through a 2.5 µm particle size separator and passed through the filter for 5 min, yielding the $PM_{2.5}$ exhaust particle samples. Three filters were reinstalled into the air sampler for repetition of each test. Once the tested fuel was changed, the tested engine was operated for at least 30 min before the samples were collected in order to avoid a possible memory effects from the previous run. All tests were run on the same engine. A field blank was run using the same procedure, except the exhaust was not collected.

Before sampling, calibration of air sampler was performed using a calibrated orifice flow rate transfer standard. The flow meter was calibrated against a laminar flow element (LFE) flow measuring device. The actual flow rate was calibrated by measured the transfer standard pressure of manometer in inches of water at different rotameter indications in the range of 4.0–6.5 L/min. Exhaust volume at standard condition, 318.2 K and 726.4 mmHg; was calculated from actual flow rate derived from air sampler calibration (Appendix B) [22].

To protect sample from moisture, the glass fiber filters were kept in a desiccators at room temperature for 24 hours before weighing. The fiber filters were then weighed using a microbalance, which was placed on an anti-vibration table, on top of a concrete bench, at 25°C and 60% relative humidity. The filters were sealed in a plastic bag and stored in the desiccators until used. To determine the net mass of particles after sampling, the filters were stored in the desiccators for 24 hours to remove moisture before weighing. The PM filter samples were stored under cool and dark conditions in refrigerator (-18 to -20 °C) until sample extraction and analysis.

2.5 Determination of physical properties of tested fuel samples

The properties of tested biodiesels including kinematic viscosity, cloud point, pour point, flash point and gross heating value were evaluated according to American Society for Testing Material (ASTM) standards method listed in Table 2.3. However, due to a limitation of the instrument, ASTM D1298 procedure was slightly modified for density test using hydrometer. The experiment was carried out at room temperature.

Hydrometer is an instrument comprised of a vertical scale inside a sealed glass tube weighted at one end. Tested fuel was transferred to hydrometer cylinder which was placed in a vertical position and free from air currents. Hydrometer was gently lowered into the fuel sample and it was then pressed about two division scales into the liquid and being released. The hydrometer scale was recorded by means of which the hydrometer floated freely away from the walls of the cylinder and then the principal surface of the liquid cut the scale.

Table 2.3 Standard method for fuel properties determination

Parameter	Unit	Reference
Kinematic viscosity at 40°C	cSt (mm^2/s)	ASTM D445
Cloud point	°C	ASTM D2500
Pour point	°C	ASTM D97
Flash point	°C	ASTM D93
Gross heating value	MJ/kg	ASTM D240

2.6 Extraction of sample

Each sample filter which contained particulate matters was cut into small pieces and put into an Erlenmeyer flask. After adding 1-NP-*d*9 as internal standard, filter samples were ultrasonically extracted twice with benzene/ethanol (3/1, v/v). After the addition of dimethysulfoxide (DMSO), the extract was placed under a stream of nitrogen until the benzene-ethanol was completely evaporated. The resulting DMSO solution was mixed with acetonitrile, and then filtered through a centrifugal filter (Ultrafree-MC, Millipore) of 0.45µm pore size [23].

2.7 HPLC for analysis of PM exhaust sample

The HPLC system, including a FCV-12AH six-port valve (Shimadzu, Kyoto, Japan), is illustrated in Figure. 2.1 and consists of a LC-10AD pump (pump 1, Shimadzu), one L-6200 pump (pump 2, Hitachi, Tokyo, Japan), a LC-10AD VP pump (pump 3, Shimadzu), a Rheodyne model 7125 injector (20_L loop), a DGU-14A degasser (Shimadzu), a 2475 fluorescence detector (Waters, Tokyo, Japan), and a CR-7A-plus integrator (Shimadzu). The system consists of five HPLC columns, a guard column (Cosmosil 5-MS-II; 4.6mm i.d.×10mm, Nacalai Tespue, Kyoto, Japan), a clean-up column (Cosmosil 5C18-MS-II; 4.6mm i.d.×50 mm), a concentration column (Spheri-5 RP-18; 4.6mm i.d.×30mm, Chemco, Tokyo, Japan), a reduction column (NPpak-RS; 4.0mm i.d.×10mm, JASCO, Tokyo, Japan) and a separation column (Cosmosil 5C18-AR-II; 3.0mm i.d.×250mm, Nacalai). The reduction column was kept at 80 ˚C in a CTO-2A column oven (Shimadzu) and the guard column, the clean up column, the concentration column, and the separation column were kept at 40 ˚C in a CTO-10AS VP column oven (Shimadzu). The mobile phases and the flow rates were as described in Figure 2.4. The excitation (λex) and emission (λem) wavelengths were 360 and 430 nm, respectively. The column-switching sequence used is shown in Table 2.4. The interfering substances which elute faster or later than analytes from the clean-up column were discarded and a fraction of only the reduced analyte and internal standard were quantitatively transferred to the separation column.

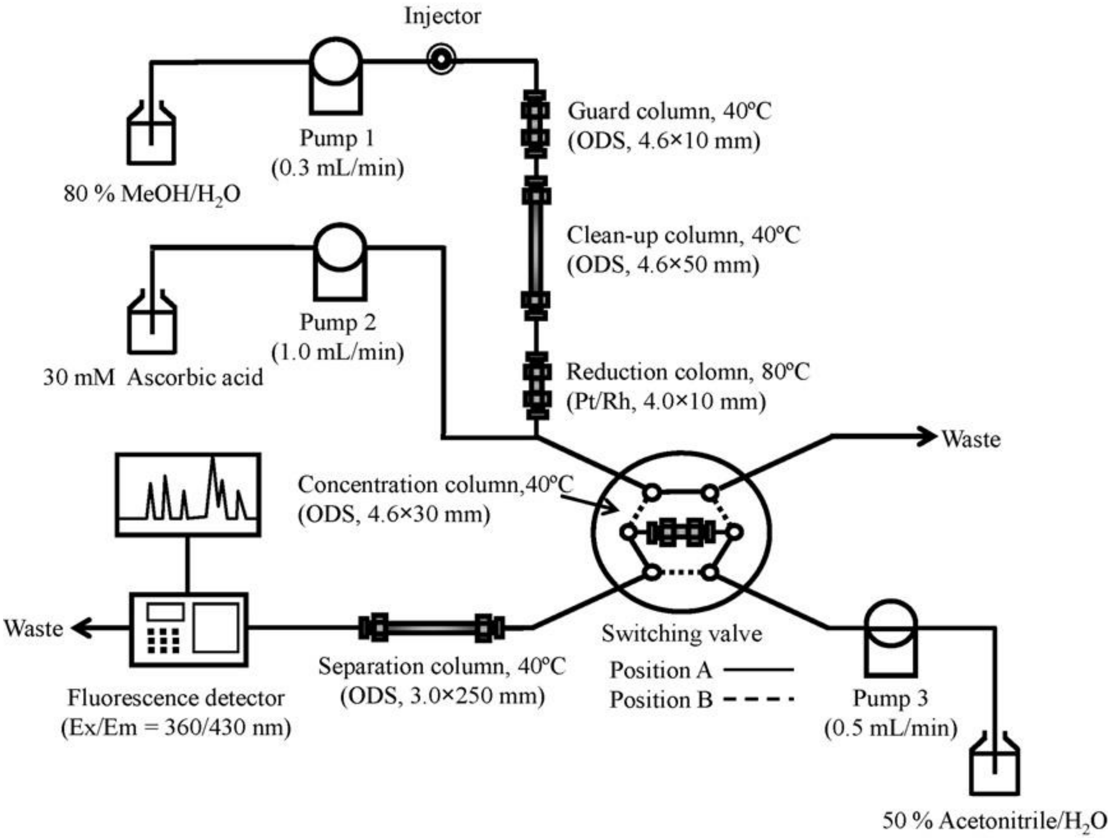

Figure 2.4 Schematic diagram of the proposed HPLC system for the determination of 1-nitropyrene [23]

The recovery of 1-NP was evaluated from the recovery of 1-NP$_{d9}$. The recovery rate of 1-NP was 82.24±11.7. The low percent recovery may affect the variation of these 1-NP emissions from the tests of various tested fuels.

Table 2.4 Analytical procedure

Step	Condition
Injection step (0 min, position A)	20 µL of the sample solution is injected
Clean-up and reduction step (0-12 min, position A)	The analysis were separated on the clean-up column from interfering substances and then reduced to the corresponding amino-derivatives by the reduction column
Trapping step (12-16 min, position B)	The reduced analysis were trapped on the concentration coloum with decreasing the content of methanol in the eluate
Detection step (16-40 min, position A)	The enriched substances were eluted and sepatared on the separation column

2.8 Data and precision analysis

1-NP emissions from the exhaust of various fuels were collected. A summary of available 1-NP emissions data are represented in Appendix C. The coefficient variations (CV) of most sample groups were relatively low (less than 40%) suggested reliability of the data presented , although the CV of CB50 groups were to be slightly high.

The results from appendix C revealed that some tests were difference from the others at significant difference; $p<0.05$. Completely randomized design (CRD) and paired sample T-test were used for statistical analysis.