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PROCESSING OF AROMA CHEMICALS AND FRAGRANCE MATERIALS

DP/VIE/86/033/11-54

SOCIALIST REPUBLIC OF VIET NAM

Technical report: Application of gas liquid chromatography*

Prepared for the Government of the Socialist Republic of Viet Nam
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Mohan L. Maheshwari,
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* This document has not been edited.

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C O N T E N T S

A B S T R A C T

Dr. Mohan L. Maheshwari, Gas Chromatograph Expert in the Project "Processing of Aroma Chemicals and Fragrance Materials at VINAROM, HoChiMinh City, Socialist Republic of Vietnam, No. DP/VIE/86/033/11-54" took up his assignment for two months (14 Oct 1991 - 13 Dec 1991). He was briefed by CTA at HoChiMinh City and had discussions with National Project Director, Deputy National Project Director, Director VINAROM and Deputy Director, VINAROM about the project.

Expert gave lectures on GC, installed columns, checked functionality of equipment and gave demonstrations on its applications in aroma chemicals and essential oils. Various parameters were developed to analyse all essential oils, their isolates, which are in the project. Trainees were examined and now they are able to analyse various samples by themselves.

Expert demonstrated separation of closely related isomers by fractionation. He also practically showed synthesis of terpenyl acetate, citronellyl acetate, citronellyl formate, citronellyl butyrate, citronellol, nerol and hydrolysis of citronella oil fraction on lab/bench scale from 100g to 10kg.

All the operational parameters, instructions, chromatograms and details of synthesis are available on the site. In addition to this expert has brought various standard specifications of different countries (ISO, EOA, French, USSR, Indian, BP. & BPC), 110-standard reference samples, library of 92-chromatograms, capillary chromatograms of 7-Vietnamese essential oils and left at site.

Trainees have learnt techniques of using equipment, synthesis and fractionation and now they can do themselves. Products prepared in the programme are of high quality and can be compared with other commercial samples. Certain suggestions are made in the recommendations, which will further improve the R & D efficiency. Essential oils, their isolates, synthesis of aroma chemicals and formulation of compounds have great potential for domestic use in Vietnam as well as for export.

R E C O M M E N D A T I O N S

In order to make best use of the project following recommendations are made from quality analysis and Research & Development point of view:

1. **For improvement in efficiency of GC analysis in future, following may be provided:**

(i)	Internal standards - alkanes (C_{10} - C_{20})	
(ii)	Solid support - Chromosorb WHP	200g
(iii)	(a) Empty glass columns - 2m x 3mm	2
	(b) Empty glass columns - 3m x 3mm	2
(iv)	(a) Nitrogen gas pressure regulator	1
	(b) Hydrogen gas pressure regulator	1
(v)	Syringes 1 microlitre	2
(vi)	Capillary column PEG 20M, 25 - 30 meters	1
(vii)	Floppy discs	20
(viii)	Sample vials with good stoppers 3ml	100
	5ml	100
	15ml	50
	25ml	50
(ix)	(a) Acetone - Analytical grade	2.5 l
	(b) n-Hexane - Analytical grade	2.5 l
(x)	Books on GC may be procured (Annex 15)	
(xi)	Shelves may be installed to keep samples in GC room	
(xii)	A refrigerator is required for preserving unstable reference samples.	
(xiii)	An electronic engineer may be identified for service and repair of GC equipment and be trained for future trouble free service.	
(xiv)	Department of standardization, metrology and quality control, who represents International Standard Organisation in Vietnam, maybe approached for recognition of VINAROM as a quality control lab.	

2. **For bench scale synthesis of aroma chemicals following should be made availble for further scaling up :**

(i) Glass Reaction flask-20 l, 5-neck, butt joints, teflon stirrer with motor (controlled), teflon stop cock in the bottom, 20l heating mantle with energy regulator, addition funnel (5l), glass column (0.8m), condenser, azeotrop head, perkin triangle, steel frame stand.

(ii) Glass Washing flask-20l-4-neck, butt joints, teflon stop cock in the bottom, teflon stirrer, jacketed bath, steel frame stand.

(iii) Heating mantle with energy regulator -1 litre -1
Heating mantle with energy regulator -2 litres -1

3. Quality of essential oils, isolates and aroma chemicals

(i) Oils of turpentine, lemongrass, Ocimum gratissimum, O. basilicum, cassia, sassafras are of high purity, meet ISO specifications, hence have very good export potential.

(ii) Alpha-terpeneol, terpenyl acetate, citronellyl acetate, citronellyl formate and citronellyl butyrate synthesised are of good quality and can be used in fragrance compounding.

(iii) Citronellol, nerol and geraniol synthesised from citronellal and citral needed further improvement in reaction conditions and work up procedure, to optimise yield.

(iv) Better strains of Eucalyptus citriodora, palmarosa, citronella, mentha etc. may be introduced in order to improve quality of oil for better utilization in industry.

(v) Cedar wood oil may be rectified further and specifications be developed.

(vi) Aloe wood distillation technology maybe improved and specifications for uniform quality be developed as this is a very high priced oil ($6000/Kg).

LIST OF ANNEXURES

I N T R O D U C T I O N

The Project "Processing of aroma chemicals and frangrance materials at VINAROM factory, HoChiMinh City, Socialist Republic of Vietnam, No. DP/VIE/86/033/11-54" was joined by Dr. Mohan L. Maheshwari, Gas Chromatograph Expert (GCE) on 14 October, 1991. Briefing was done by Dr. C. K. Atal, CTA. GCE had discussions with Mr. Dinh Van Thin, National Project Director (NPD), Dr. Do Linh Cuong, Deputy National Project Director, Mr. Hoang Quang Lap, Project Secretary, Mr. Le Muoi, Director, VINAROM Factory, Mr. Nguyen Van Quy, Deputy Director, VINAROM Factory and Project Co-ordinator about the Project. GCE was taken around VINAROM Factory and R & D and production facilities were shown to him.

Earlier a project entitled "Processing of Vietnamese Essential Oils and Related Natural Products - DP/VIE/84/010" was provided by UNIDO for Research and Development and marketing. As a result of which new prospective essential oils are now available and there is a dire need for producing fragrance materials from them for domestic use . Hence this second project has been provided through agency of UNDP - UNIDO. The project involves fractionation of locally available essential oils and production of basic array of aroma chemicals, involving synthetic transformations also. Fragrance chemicals are to be produced first on laboratory scale and then to be scaled up in pilot plant. These aroma chemicals are to be used for formulation of compounds to serve needs of ongoing detergents, soap, cosmetic and toiletries industries. These will also have export potential to earn foreign excahnge after meeting domestic demand.

This programme needed development of a good analytical laboratory and training of national staff. Gas chromatography is very much essential for quality evaluation of fragrance materials. A computerized Gas chromatograph with integrator (Shimadzu Model GC-9 APTF) was provided in the project.

DUTIES:

The expert was expected to work under the direct spervision of the CTA and NPD as shown in Job Description (Annexure - 1) for two man months (14 Oct 1991 - 13 Dec 1991). Expert has carried out work based on Job Description. He was also asked to demonstrate separation of some aroma chemicals as well as synthesis of some fragrance chemicals and monitoring course of chemical reactions by GC analysis. List of trainees and staff who worked with expert is given in Annexure - 2.

As requested by site, expert brought standard documents on desired essential oils and their isolates. These included 19 International Standards (ISO: Annexure - 3), 27 - Essential Oil Association of U.S.A. Specifications (Annexure - 4), 1 - French Standard & 1 - Russian Standard (Annexure - 5), 21 - Indian Standards (Annexure - 6), 8 - British Pharmacopoea specifications (Annexure - 7), 10 - British Pharmaceutical Codex specifications (Annexure - 8). He also brought 110 - Reference Standard samples of essential oils, isolates, and other aroma chemicals for identification and analysis of essential oils and aroma chemicals in the project (Annexure - 9). Library of 92 Gas chromatograms of essential oils, isolates and various aroma chemicals, prepared in Expert's laboratory and brought to the site (Annexure - 10). He also brought capillary GCs of 7 - essential oils of Vietnam origin (Annexure - 11). All these materials are kept at project site. All the assigned work was completed by expert. Salient features of the work done during the mission of the expert are given in the following pages.

I. GAS LIQUID CHROMATOGRAPHY (GC)

A. Theory.

Eight lectures were given to trainees as shown in the following table:

Subject	No. of lectures
. Principles of GC	1
. Maintenance of GC	1
. Column packing	1
. Isothermal/temperature programming and analysis of essential oils/isolates	3
. Quantitative analysis on packed column	1
. Capillary columns and analysis of essential oils	1

B. Practicals.

A Gas chromatograph-GC 9 APTF of Shimadzu (Japan) make has been provided with Integrator C-R6A chromatopac, computer with Floppy Disk Drive, Expansion case C-R 3A and accessories in the project. The basic equipment was initially installed with the help of person who worked in earlier Project (DP/VIE/84/010). GC instrument was checked up by Expert thoroughly, two columns Carbowax 20M (3.1m x 3mm) and SE-30 (3.1m x 3mm) polar and nonpolar were installed and conditioned them. Startup, maintanance and closing of GC equipment was demonstrated. Efficiency of columns was checked by using standard compounds. Instructions for daily operation of instrument were prepared (Annexure-12). GC analysis of various essential oils of

Vietnam as wellas of standard reference compounds and isolates were carried out. Different sets of parameters were developed for different oils, isothermally and with temperature programming. All these chromatograms as shown in Annexure-13 are kept at the project site for comparison in future. Important features of analysis results are as follows:

1. Analysis on polar Carbowax 20M column:

1.1 Turpentine oil:

G.C. composition was found as shown below:

	%		%
Alpha-pinene	76.66		
Camphene	0.92	Fenchyl alcohol	1.78
Beta-pinene	1.09	Borneol	0.96
Delta-3-Carene	0.69	Alpha-terpeneol	3.19
Limonene	1.78	High boilers	6.89
p-Cymene	0.43		

This is a good source of alpha-pinene and many related products like terpeneol, camphor, isobornyl acetate etc. can be synthesised from this.

1.2 Terpeneols:

Terpeneol prepared from turpentine oil via terpin hydrate was a mixture of following:

	%		%
Terpenes/low boilers:	21.88	Beta-trans-terpeneol:	11.18
Terpenene-1-ol :	3.67	Beta-cis-terpeneol :	2.75
Terpenene-4-ol :	1.02	Alpha-terpeneol :	58.63

1.3 Citronella oil:

Composition is given below:

	%		%
Low boilers	4.13	Geranyl formate	0.54
Citronellal	35.67	Citral-a	2.72
Linalool	1.46	Citronellol+geranyl	
Isopulegol	1.60	acetate	13.51
Beta-elemene	2.09	Citronellyl butyrate	2.35
Citronellyl formate	0.26	Nerol	0.30
Citronellyl acetate	2.87	Geraniol	21.49
Citral-b	1.09	High boilers	7.36

Main difference between this oil and oil from Java was observed by presence of isopulegol and citral.

1.4 Eucalyptus citriodora oil:

Composition

	%		%
Low boilers	4.94	Citronellyl acetate	1.28
Citronellal	59.59	Citronellol	12.00
Linalool	0.20	Geraniol	0.97
Isopulegol	11.26	High boilers	7.52
Beta-elemene	1.65		

This oil is a good source of citronellal but also having isopulegol in high amounts.

1.5 Lemon grass oil:

Composition

		%			%
Low boilers	:	0.50	Neral (Citral-b)	:	36.34
Methyl heptenone	:	1.97	Geranial (citral-a)	:	48.30
Citronellal	:	0.20	Citronellol	:	1.35
Linalool	:	0.68	Nerol	:	2.81
Linalyl acetate	:	0.69	Geraniol	:	3.05
Isopulegol	:	1.34	High boilers	:	0.34
Beta-elemene	:	1.35			

This oil has quite high content of citral (84%) and minimum amount of sesquiterpene hydrocarbons, which often cause difficulty in fractionation of lemon grass oil, if present in larger amount. Citral from this oil will find variety of uses in perfumery/flavour and synthesis.

1.6 Litsea cubeba oil:

Two oils were analysed:

Components	L. cubeba %	
	SVDC	Enteroil
Thujene	0.16	0.14
Alpha-pinene	0.36	0.55
Beta-pinene	1.77	1.18
Limonene	2.96	4.07
1:8-cineole	1.16	1.61
Methyl heptenone	2.23	3.09
Aldehyde C-9	1.09	0.12
Citronellal	13.03	3.08
Linalool	2.43	9.57
Isopulegol	2.71	1.69
Beta-elemene	1.30	1.15
Caryophyllene	2.72	0.86
Neral	20.42	27.14

Geranial	31.39	38.96
Citronellol	3.98	1.46
Nerol	0.84	0.72
Geraniol	1.14	2.27
High boilers	4.04	1.33

L. cubeba oil from Enteroil has higher content of citral (66.13%) than that of SVDC (50.81%) later sample has higher content of citronellal (13.03%), lesser amount of linalool and higher amount of caryophyllene (2.72) than those of Enteroil.

1.7 Palmarosa oil:

Composition

	%		%
Low boilers	1.16	Geranyl acetate	10.63
Citronellal	0.58	Nerol	0.55
Linalool	2.48		
Isopulegol	0.44	Geraniol	65.92
Beta-elemene	1.21		
Citronellyl formate	0.58	Geranyl butyrate	2.37
Caryophyllene	3.50	Geranyl isovalerate	0.94
Citronellyl acetate	0.83	Geranyl hexanoate	2.37
Geranyl formate	1.76	Geranyl caproate	1.44
Neryl acetate	4.30	High boilers	0.38

Palmarose oil is basically having all the characteristics but in addition to this it has citronellal (0.58%), isopulegol (0.44%, small quantities of citrtonellyl acetate and formate, hence there is little change in the odour. New materials are also being grown now.

1.8 Cajeput oil:

Composition

	%		%
Alpha-pinene	1.78	Linalool	3.69
Beta-pinene	1.20	Trans-beta-terpeneol	1.06
Sabinene	0.91	Cis-beta-terpeneol	0.57
Myrcene	0.31	Alpha-terpeneol	10.48
Limonene	2.80	High boilers	8.73
Cineole (1:8)	63.00		
Gamma-terpinene	0.26		
p-Cymene	2.21		

Cajeput oil has high percentage of cineole followed by alpha-terpeneol.

1.9 Mentha arvensis oil:

Composition

		%			%
Alpha pinene	:	0.84	Menthone	:	18.17
Beta-pinene	:	0.63	Isomenthone	:	3.51
			Linalool	:	0.70
Sabinene	:	0.14	Menthyl acetate	:	3.68
			Neo menthol	:	2.06
Limonene	:	1.13	Caryophyllene	:	0.71
Cineole (1:8)	:	0.64	l-menthol	:	52.59
Gamma terpinene	:	0.20	Isomenthol	:	1.43
p-Cymene	:	1.07	Pulegone	:	0.93
			Piperitone	:	1.06
Octanol-3		0.26	carvone	:	8.36
			High boilers	:	1.71

M.arvensis oil has low content of menthol (52.59%) and higher content of menthone (18.17%) and carvone (8.36%). After little rectification oil can be used as dementholised oil.

1.10. Orange oil:

Composition

		%			%
Alpha-pinene	:	0.48	Beta-elemene	:	3.48
Limonene	:	63.55	Unidentified	:	1.63
Aldehyde C-8	:	0.12	Neral	:	2.26
Aldehyde C-9	:	0.03	Geranial	:	5.42
Linalool	:	0.31	Nerol	:	1.23
Linalyl acetate	:	0.72	Unidentified	:	2.88
Isopulegol	:	0.17	Geraniol	:	2.11
			High boilers	:	14.06

This oil has limonene content of 63.55% and can find use in flavour.

2. Analysis on non-polar SE-30 column:

2.1 Ocimum gratissimum oil: This was analysed on GC, compared with GC of similar oil from India and checked with reference compounds.

The composition is as follows:

		%			%
Benzaldehyde	:	0.23	Trans-iso eugnol/		
Beta-cis-Ocimene		3.83	Caryophyllene	:	4.76
Methyl chavicol	:	1.27	Alpha-humulene	:	0.51

Eugenol		70.00	Eugenyl acetate	:	4.93
Vanillin	:	0.79	Unidentified	:	2.51
Cis-isoeugenol/ methyl eugenol	:	2.21	High boilers	:	3.57
Beta-elemene		1.03			

This oil has very good source of eugenol and can be used in place of eugenol ex. clove oil for perfumery, flavour and synthesis of iso-eugenol, eugenyl acetate etc.

2.2 Ocimum basilicum oil:

Composition

		%			%
Benzaldehyde	:	0.12	Eugenol	:	0.13
Beta-cis-ocimene	:	1.99	Methyl eugenol	:	0.64
Linalool	:	0.79	Beta-elemene	:	0.41
Terpin-1-ene-4-ol	:	0.93	Caryophyllene	:	1.98
Methyl chavicol	:	86.21	Eugenyl acetate	:	0.38
Anisaldehyde	:	0.16	Unidentified	:	0.52
Cis-anethole	:	0.53	Unidentified	:	1.52
Trans-anethole	:	0.55	High boilers	:	1.78

This is very rich in methyl chavicol (86%). GC composition was also compared with those of Madagaskar, Indian and Egyptian oils. This oil compared well with those of Madagaskar and Comores Islands and can find good use in perfume formulations, synthesis of trans-anethole from natural source, as well as for export.

2.3 Star anise oil:

Composition of oil is as follows:

		%			%
Benzaldehyde	:	0.31	Trans-anethole	:	81.41
Cis-beta-ocimene	:	2.96	Eugenol	:	0.28
Linalool	:	0.33	Terpinyl acetate	:	3.77
Methyl chavicol	:	3.13	Caryophyllene	:	0.43
Cis-anethole	:	3.54	High boilers	:	2.86

This oil is a good source of natural trans-anethole required for flavour/perfume formulations. Natural trans-anethole is perferred over synthetic t-anethole.

2.4 Cinnamomum cassia oil: Cassia oil produced from its bark was analysed on GC, compared with those of C. zeylenicum and standard reference samples. Its composition is as follows:

	%		%
Low boilers	3.45	Cinnamic alcohol	0.26
Cinnamic aldehyde	90.55	Methyl eugenol	0.15
Unidentified	0.18	Cinnamyl acetate	2.29
Eugenol	0.17	High boilers	2.62

Oil has high prcentage of cinnamic aldehyde and meets ISO-specifications.

2.5 Sassafras oil: Oil is mainly constituted of safrole (96%) as given below and hence a good source of safrole, which can be used for preparation of isosafrole, heliotropine and piperonal butoxide (a synergist for insecticides):

	%		%
Low boilers	0.39	Safrole	95.76
Unidentified (heliotropine?)	1.49	High boilers	2.29

2.6 Cedarwood oil: Two kinds of oils were run on GC and analysed for following components:

	Yellow colored (%)	Dark brown colored (%)
Low boilers	0.66	0.60
Sesquiterpene hydrocarbons	60.23	54.72
Carbonyl compds.	11.15	10.41
Sesq. alcohol	22.19	25.90
High boilers	5.76	8.28

Basically, there is not much difference in two oils, but they require rectification for improving its woody odour. Compounds could not be identified due to non availability of atlantone, cedrol and cedrenes.

2.7 Vetiver oil: Oil samples of different origins (Indian - 2-cultivated 1-wild, Indonesian). Vetiverol and vetiveryl acetate were analysed on GC, compared with those present in library brought by expert (Indian, Indonesian, Reunion, Haiti etc.). the differences in GCs were explained to trainees and chemomarkers of oils were shown. Two-three types of vetiver oils will soon be available in Vietnam.

2.8 Aloe wood oil: The wood of Aquilaria cresna Pierre (family - Thymeliaceae), after mechanical removal of resin, gives 3-fractions of oil on steam distillation, whose price varies from US Dollars 2,000 to 6,000 per kg. GC patterns of these fractions were developed-Fraction-1 has 34% low boilers and large number of peaks are shown by rest of the portion. Fraction-2 shows less complicated GC (6-major components), low boilers are almost absent, some of the components are common with fraction-1. Fraction-3 (highest priced) has a major component (15.56%) in carbonyl region, about 7 other main components (3 to 9.7%) in the sesquiterpenic alcohol region and few components are common with fraction-2. It appeared that there are some artefacts forming in fractions-2 and 3 during high pressure steam distillation.

This oil appears to be some what different than oil from Aquilaria agallocha Roxb (agar wood), growing in North-East Region of India. Only a few compounds were reported from agar wood many years ago, reference components are not available. These three patterns can be standardised for gradation of aloe wood oil.

2.9 Fokienna hodginsii oil: GC of this oil showed very little percentages of low boilers and mostly C15 and higher components. This is new oil having fatty (waxy), woody spicy with slight citrus odour and components are not known. Components in GC are in the region where sesquiterpene ketones/aldehydes show up. This oil needs further work on its utilization.

3. A demonstration was given for packing GC columns.

4. Quantitative analysis - using internal standard:

A demonstration was given for calculation of Response factor (K) of reference substance with respect to internal standard by formula given below:

$$K = \frac{AE \times mR}{AR \times mE}$$

AE = Peak area in integrator units of internal standard

AR = Peak area in integrator units of reference substance (to be estimated)

mR = Mass in mg. of reference substance.
mE = Mass in mg. of internal standard.

Content of reference substance in essential oil was calculated by following expression:

$$Cx = \frac{Ax \times mE \times K}{AE \times m} \times 100$$

Cx = Content expressed as prcentage by mass

Ax = Peak area of reference constituent in integrator units

AE = Peak area of internal standard in integrator units

m = Mass in mgs. of the essential oil

mE= Mass in mgs. of the internal standard

K = Response factor calculated from earlier formula

5. Test for GC training:

A question paper was set for the trainees (Annexure-14). This paper consisted of 20-questions, which covered all theoretical and practical aspects of knowledge for GC use. Except a few minor mistakes trainees have fared well in the examination.

6. Books for Gas chromatography:

A list of books (7) has been prepared and suggested for use in library(Annexure-15). Last 3-books are highly useful in essential oil analysis work.

II. ISOLATES AND SYNTHESIS OF AROMA CHEMICALS.

All the precesses were monitored by GC. analysis.

1. **Isolation of alpha-terpeneol:** Terpeneol prepared from turpentine oil via terpin hydrate was a mixture of terpenes (22%) and terpeneol isomers (77%). Percentage of alpha-terpeneol was 58% in the mixture. Parameters were developed for fractional distillation of 14Kg mixture under vacuum as given below:

Pot temperature	:	112° - 123°C
Reflux temperature	:	78° - 97°C
Vacuum	:	25 - 9 mm

Perfumery grade alpha-terpeneol (95%) was obtained as 30% of mixture. Alpha-terpeneol of second grade (88%) was 10% of mixture used for synthesis of terpenyl acetate. Remaining terpeneol mixture (initial cuts) was for general use in toiletories and soap.

2. **Synthesis of terpenyl acetate:**

Terpeneol was acetylated with acetic anhydride at 30°C in 4-batches. First conditions were standardised on small amounts then scaled up to 10Kg.

Batch No.	Terpeneol	Acetic anhydride	Time (hr)	Conversion %
1	100 g	84 g	20	98
2	700 g	567 g	24	99
3	2.1 Kg	1.7 Kg	30	99
4	10.0 Kg	8.3 Kg	24	98

Terpenyl acetate formed is of good quality having good clean woody odour. Its GC gives much better picture on comparison with standard commercial sample.

3. **Synthesis of citronellyl acetate:**

Like synthesis of terpenyl acetate, synthesis of citronelyl acetate was also demonstrated on one batch only. Citronellol (100g) was reacted with acetic anhydride (54g) at 30°C for 5hr. Monitoring on GC showed presence of 6% unreacted citronellol. Reaction was continued for another 17hr. Conversion was complete and product formed, compared well with standard sample in GC and odour.

4. Synthesis of citronellyl formate:

Citronellol was reacted with formic acid in two batches as given below:

Batch No.	citronellol	formic acid	Reaction time & temp.	Conversion
1	20 g	16 g	7hr(40°C)	95 %
2	7 Kg	5.6 Kg	14hr(40°C)	95 %

Citronellyl formate formed has good sweet floral rosaceous odour and sample is much better than commercial sample.

5. Synthesis of citronellyl butyrate:

Citronellol (109g) was reacted with butyric acid (74g) in benzene for 8hr. Water (8g) was removed azeotropically. Gc showed 98% conversion into citronellyl butyrate. On working up the product was found to have clean odour of citronellyl butyrate.

6. Hydrolysis of citronella oil fraction after removal of citronellal:

Citronella oil was fractionated to remove citronellal (82.42%) having small amounts of linalool (2.70%), isopulegol (6.36%) and citronellyl acetate (3.62%). The remaining portion (200g) was hydrolysed at 100°C with 10% NaOH for 8hr. All esters of citronellol and geraniol hydrolysed. GC was very clean and showed presence of mainly citronellol (20.53%), nerol (3.78%) and geraniol (70.19%) with minor amount of beta-elemene (3.48%). This material served a good starting material for isolation of citronellol and geraniol by fractional distillation.

7. Synthesis of citronellol:

7.1 Reduction of citronellal in isopropanol was carried out for 6hr by using freshly prepared aluminium isopropoxide from 150g of aluminium, mercuric chloride (2.5g), carbon tetrachloride (11.5g) and isopropanol (1325ml) in 3-batches as given in following table:

Batch No.	Starting material	wt. of material (kg)	Conversion (%)
1	Citronellal ex. Java citronella oil	1.5	90
2	-do-	1.7	90
3	-do-	3.4	99
4	Citronellal ex. Eucalyptus citriodora	3.8	90

In batches 1-3 citronellal was 80.4% pure (GC) and rest of components were isopulegol (4.9%), citronellol (3%) and geraniol (1.6%). The product formed had citronellol (83.3%) and isopulegol (3.73%). In batch-4, citronellal used was 75.78% pure and other compounds were isopulegol (21.08%) and beta-elemene (1.20%). The product formed had citronellol (66%), isopulegol (24.49%), beta-elemene (0.8%) and high boilers (3.3%).

In first three batches conversion was good (GC), in third batch efficiency of reaction could be improved to more than double. But in batch No. 4 due to presence of high amounts of isopulegol, citronellol percentage decreased.

7.2 A bigger batch of citronellal (19.6Kg) ex. Java citronella having citronellal only 53.89% and rest linalool (3.1%), isopulegol (16.99%), beta-elemene (1.66%), citronellol (7.9%), nerol (0.8%) and geraniol (6.2%) was reduced with aluminium isopropoxide prepared from 400g of aluminium. composition of product was as follows: linalool (3.29%), isopulegol (19.30%), beta-elemene (2.11%), citronellol (58.51%), nerol (1.45%) and geraniol (5.6%).

In the bigger batch 30% material got polymerised due to high heat generated during decomposition of complex by sulphuric acid. Hence it was concluded that starting material with better purity should be used and during decomposition of complex cooling at low temperature is essential.

8. Synthesis of nerol:

Based on the experience in section-7, study was made on reduction of 2.7 Kg citral -'a' and -'b'(90%) with aluminium isoproxide made from 104g of aluminium. aliquots were drawn from reaction mixture at different duration and decomposition was done at different temperatures as given below:

Duration of reaction (hr)	Complex decomposition temp. (°C)	% Nerol + geraniol content in product
5	3 - 5	85
5	50	80
8	3 - 5	84
48	30	62

It is concluded from above results that reduction of citral is to be stopped at 5 hours and decomposition should be done at low temperatures (3-5°C) to get better quality nerol and geraniol.

It was suggested to use distilled aluminum isopropoxide and low temperature for decomposition in-order to avoid polymerisation. Therefore nerol was prepared using these modifications to get better quality product and minimum residue after distillation..

III. CONCLUSION

Gas chromatography Expert in his assignment for 2 man months has completed job requirements like - giving lectures on GC (covering all the aspects), demonstration of its usefulness, specially for quality control of essential oils, isolates and for production of synthetic aroma chemicals. He has given practical training for analysis of essential oils of Vietnam, their isolates and reference compounds. He has also prepared procedures for maintenance, starting and closing of GC equipment. Column packing and quantitative analysis have been demonstrated. A test was conducted in which trainees have faired very well.

Expert was also assigned work on isolation and synthesis of certain aroma chemicals. He has demonstrated practically isolation of terpeneols for perfumery, synthesis of terpenyl acetate, citronellyl acetate, citronellyl formate, citronellyl butyrate, citronellol, nerol and improving quality of citronellol and geraniol from citronella oil.

Trainees have picked up the basic knowledge on GC and its practical use. Now they are using the equipment by themselves by analysing essential oils, isolates, monitoring organic reactions during synthesis and analysing final products. They have learnt the techniques and conditions for carrying out synthesis of earlier mentioned products and they have reproduced these results on lab bench-scale. The products prepared are of high quality and can match with good quality materials from else where. GC machine is working very well and all the essential accessories are there. In the recommendations some additional items like books, spares, lab. wares and equipments are suggested, which will further improve the R & D efficiency. It is certain that essentiaL oils, their isolates, synthesis of aroma chemicals and formulation of fragrance compounds have very good future in Vietnam for their domestic use as well as for export.

ANNEXURE 1

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
UNIDO
JOB DESCRIPTION DP/VIE/86/033/11-54

POST TITLE : Gas chromatograph Expert

DURATION : 2 m/m

DATE REQUIRED : April - May 1991

DUTY STATION : HoChiMinh City

PURPOSE OF PROJECT : Utilization of indigenous essential oils to develop suitable fragrance materials and formulations for local industry as well as export.

DUTIES: Under the UNIDO Project, a computerized GC with integrator (shimadzu Model GC-9APTF) has been purchased. The expert will carry out the following work under the supervision of the CTA:-

1. Lectures/Discussions on theory and application of Gas Liquid Chromatography.

2. Full commissioning of the instrument and demonstration of its usefulness for quality control of essential oils.

3. Demonstration of methods of quantitative GLC analysis.

4. Use of reference standards and calibration.

5. Column packing and mathods of packing.

6. Monitoring course of a reaction and its optimisation by GLC analysis.

7. Any other aroma chemical related work assigned by the CTA.

The expert will also furnish a completed and fully processed terminal report on the work done by him at the completion of his mission outlining the findings and his recommendations for follow-up action.

Qualifications:

Chemist, Pharmacist with considerable experience in R & D of analytical chemistry and essential oils, theoretical and practical experience in the utilisation of analytical instruments, especially gas chromatography.

Language : English/French

Background Information:

Vietnam has been a producer of natural essential oils but in recent times this industry has suffered through want of Research and development back-up and modern marketing initiatives. To remedy the situation the government through the agency of UNDP and UNIDO has provided the industry with R & D backup in the form of a; project titled: Processing of Vietnamese essential oils and related natural products, DP/VIE/84/010.Since new prospective essential oils will soon become available and a dire need for fragrance materials exist the government again through the agency of UNDP-UNIDO wishes to set up this second project directed towards fractionation of locally produced essential oils and production of a basic array of aroma chemicals in order to formulate and compound fragrance materials. These fragrance materials are to be produced first on a bench scale and once the technological problems are surmounted produced on a pilot scale to serve the needs of an on-going soap and toiletries industry. Export possibilities will also be assessed to provide a potential foreign exchange earning capacity.

ANNEXURE 2

LIST OF TRAINEES

1.	Mrs. Luc Thi Van Hien	Analysis Department, VINAROM, SVDC
2.	Mr. Vu Van Chuong	-id-
3.	Miss Truong Thi Phuong	Distillation Department VINAROM, SVDC
4.	Mr. Nguyen Minh Hung	-id-
5.	Mr. Nguyen Quang Hien	-id-
6.	Mrs. Vu Bao Dung	Fragrance Department
7.	Mrs. Vo Thi Hoa	Analysis Department Vietnam Soap Factory. SVDC
8.	Miss Le Thi Thu Hoai	-id-

ANNEXURE 3

INTERNATIONAL STANDARD (ISO) TC 54

	Reference	Ed.	Pages	Title
1.	ISO 3848-1976	1	2	Oil of Java citronella
2.	ISO 3214-1974	1	2	Oil of Litsea cubeba
3.	ISO 3216-1974	1	2	Oil of Cassia
4.	ISO 3524-1977	1	2	Oil of Cinnamon leaf
5.	ISO 3475-1975	1	2	Oil of aniseed
6.	ISO 4718-1981	1	2	Oil of lemongrass(Cymbopogon flexuosus)
7.	ISO 3217-1974	1	2	Oil of lemon grass (Cymbopogon citratus)
8.	ISO 3757-1978	1	2	Oil of patchouli
9.	ISO 4716 Draft	In CIrculation		Oil of Vetiver (Vetiveria zizanioides Linnaeus Nash)
10.	ISO 9776 Draft	In Circulation		Oil of Mentha arvensis, partially dementholized (Mentha arvensis Linnaeus var. Piperscens Mallinvaud, var. glabrata Holmes
11.	ISO 11020 C.D.	to be printed in 1993.		Essential oil of turpentine
12.	ISO 1586	C.D. to be printed in 1993		Oils of Melaleuca ssp. Melaleuca alternifolia, Melaleuca, linariifolia, Melaleuca dissitiflora, type terpineol-1-ol-4
13.	ISO 1452 Draft	in Circulation		Essential oils of cedarwood, China (Cupressus funebris Endicher)
14.	ISO 1202-1981	1	3	Essential oils-Determination of 1,8-cineole content
15.	ISO 1279-1984	2	3	Essential oils - Determination of carbonyl value - Hydroxylammonium chloride method.

16.	ISO 7611-1985	1	5	Oils of lemon and petitgrain citronnier, and oil of lime obtained by mechanical process-Determination of Citral (neral + geranial) content - Gas Chromatographic method on capillary columns.
17.	ISO 7734 Draf in Circulation			Oils of Eucalyptus citriodora, geranium and citronella - Determination of citronellol and geraniol - Gas Chromatographic method on packed or capillary columns.
18.	ISO 7359-1985	1	7	Essential oils-Analysis by gas chromatography on packed columns-General method.
19.	ISO 7609-1985	1	7	Essential oils - Analysis by gas chromatography on capillary columns - General method.

ANNEXURE 4

SPECIFICATIONS OF ESSENTIAL OIL ASSOCIATION OF U.S.A.(E.O.A.)

S.No.	(Sec.II) Essential Oils	Number Specifications
1.	Basil Reunion, Oil	120
2.	Cajeput, Oil	22
3.	Palmarosa, Oil	29
4.	Patchouly, Oil	23
5.	Pinus sylvestris oil (Oil Scotch pine) (Oil turpentine Russian)	133
6.	Turpentine Rectified, Oil	252
7.	Vetiver Oil	24
	Oil vetiver Java, Reunion (Bourbon), Haiti	

	(Sec.VI) Aromatic Chemicals & Isolates	Number Specifications
8.	Cinnamic aldehyde	204
9.	Citral pure	15
10.	Citronellal	227
11.	Citronellol	17
12.	Citronellyl acetate	125
13.	Eucalyptol (1,8-Cineole)	288
14.	Geraniol	16
15.	Geranyl acetate	11
16.	Geranyl formate	162
17.	Hydroxy citronellal	5
18.	Ionones (Alpha, Beta & Gamma)	61
19.	Linalool (Synthetic)	226
20.	Terpeneol	8

ANNEXURE 5

FRENCH STANDARD

Essential Oil	Specification Number
Basil, Oil-methyl - Chavicol type (*Ocimum basilicum* Linnaeus)	NFT 75-357

RUSSIAN STANDARD

Essential Oil	Specification Number
Ocimum gratissimum , oil	GOST 9361-60

ANNEXURE 6

INDIAN STANDARDS

(Natural and Synthetic Perfumery Materials Sectional Committee PCD 18)

S.No.	IS No.	Title	Amendments
1.	326 : 1968	Methods of sampling and test for natural and synthetic perfumery materials	
2.	326(Part 6): 1986	Same as above. Determination of solubility in ethanol	Revised
3.	326(Part 11): 1986	Same as in 1. Determination of carbonyl value and content of carbonyl compounds.	Revised
4.	327 : 1961	Oil of lemon grass	Draft approved'90
5.	512 : 1988	Oil of citronella	
6.	526 : 1988	Oil of palmarosa	
7.	528 : 1989	Oil of Mentha arvensis	
8.	533 : 1973	Gum spirit of turpentinc (oil of turpentinc)	DOC.in circulation 1990
9.	553 : 1984	Rosin (gum rosin)	
10.	1799 : 1981	Citral	
11.	1800 : 1989	Geraniol	
12.	1801 : 1988	Citronellol	
13.	3123 : 1980	Hydroxy citronellal	
14.	3124 : 1975	Terpeneol	Rev.DOC.in circulation'90
15.	3134 : 1965	Menthol	Rev.Doc.in circulation'90
16.	3241 : 1985	Geranyl acetate	
17.	3250 : 1982	Methyl ionone	

18.	3925 : 1980	Eugenol	
19.	5757 : 1971	Pine Oil	Rev. DOC. in circulation'90
20.	6699 : 1989	Cinnamon leaf oil	
21.	9784 : 1981	Tejpat leaf oil	

ANNEXURE 7

BRITISH PHARMACOPOEA 1980 VOLUME I

S.No.	Name of item	Page No.
1.	Anise oil (Aniseed oil)	36-37
2.	Aniseed	37
3.	Cinnamon (Bark; Ceylon Cinnamon)	113-114
4.	Cinnamon Oil	114
5.	Eucalyptus Oil	189
6.	Euugenol	189-190
7.	Terpineol	444
8.	Turpentine oil	469

ANNEXURE

BRITISH PHARMACEUTICAL CODEX 1973

S.No.	Name of Item	Page No.
1.	Anise Oil	30
2.	Cajuput Oil	64
3.	Cineole	112-113
4.	Cinnamon	113-114
5.	Cinnamon Oil	114
6.	Citronella Oil	115
7.	Eucalyptus Oil	193
8.	Eugenol	193
9.	Terpineol	494
10.	Turpentine Oil	524-525

ANNEXURE 9

LIST OF STANDARD CHEMICALS BROUGHT FOR PROJECT

1. Aldehyde C-8
2. Aldehyde C-9
3. Aldehyde C-10
4. Aldehyde C-12
5. Anethole
6. Anise oil (DAB)
7. Anise oil (NBPGR)
8. Benzaldehyde
9. Benzyl acetate
10. Benzyl alcohol
11. Borneol
12. Camphor
13. Carene delta - 3
14. Carvone - L
15. Caryophyllene
16. Cineole 1-8
17. Cinnamon leaf oil (S.H.K)
18. Cinnamon leaf oil (DAB)
19. Cinnamic alcohol
20. Cinnamic aldehyde
21. Cinnamyl acetate
22. Cis-3-hexenol
23. Citral
24. Citronella oil (HYDBD.)
25. Citronella oil (S.H.K.)
26. Citronellal
27. Citronellol
28. Citronellyl formate
29. Citronellyl acetate
30. Citronellyl butyrate
31. Clocimum oil
32. Carvone-D
33. Dementholised oil
34. Eucalyptus oil
35. Eugenol
36. Eugenyl acetate
37. Geraniol (S.H.K.)
38. Geraniol (Ex.palmarosa oil)
39. Geraniol(Ex.citronella oil)
40. Geranium oil (S.H.K.)
41. Geranium oil (HYDBD.)
42. Geranyl acetate (S.H.K.)
43. Geranyl acetate (NBPGR)
44. Geranyl butyrate
45. Geranyl caproate
46. Geranyl formate
47. Geranyl iso-valerate
48. Ginger oil

49. Hydroxycitronellal (Extra)
50. Hydroxy citronellal (pure)
51. Ionone-Alpha
52. Ionone-Beta
53. Iso-borneol
54. Iso-bornyl acetate
55. Iso-eugenol
56. Jamrosa oil
57. Lemon grass oil CKP-25)
58. Lemon-grass oil (Pemba)
59. Lemon grass oil LS-48)
60. Lemon grass oil (S.H.K.)
61. Limonene
62. Linalool
63. Linalyl acetate
64. Methyl amyl ketone
65. Methyl chavicol
66. Methyl cinnamate
67. Methyl eugenol
68. Methyl ionone
69. Menthofuran
70. Mentha arvensis-shivalik
71. Mentha piperita
72. Mentha spicata
73. Menthone/isomenthone
74. Menthol
75. Menthyl acetate
76. Methyl salicylate
77. Nerol
78. Ocimene Beta (Cis & Trans)
79. Ocimum basilicum (Egypt)
80. Ocimum basilicum (Madagaskar)
81. Ocimum basilicum EC-291415 - Methyl chavicol type)
82. Ocimum basilicum (0095, NBPGR, Methyl cinnamate type)
83. Ocimum basilicum (Ec-174526 NBPGR Linalool type)
84. Ocimum basilicum (EC-282721 NBPGR Eugenol - Methyl Chavicol type)
85. Ocimum basilicum (Indian basil)

86. Ocimum gratissimum
87. Palmarosa oil
88. Patchouli oil (NBPGR - Indonesian variety)
89. Patchouli oil (S.H.K)
90. Patchouli oil (IIHR-Bangalore)
91. Perilla Aldehyde
92. Petitgrain oil
93. Pine oil
94. Pinene - Alpha
95. Pinene - Beta
96. Piperitone
97. Sandalwood oil
98. Turpentine oil
99. Terpinene - 1 - Ol-4
100. Terpinene - Alpha
101. Terpinyl acetate
102. Terpineol - Alpha
103. Thymol
104. Vanillin
105. Vetiver oil (S.H.K)
106. Vetiver oil(South Indian)
107. Vetiver oil (Indonesian)
108. Vetiver oil (NC-66403, North Indian)
109. Vetiverol
110. Vetiveryl acetate.

ANNEXURE 10

LIBRARY OF 92 CHROMATOGRAMS OF ESSENTIAL OILS, ISOLATES AND VARIOUS AROMA CHEMICALS BROUGHT BY EXPERT

COLUMN CARBOWAX 20M:

1. Aldehyde C-8
2. Aldehyde C-9
3. Aldehyde C-10
4. Aldehyde C-12
5. Anise oil
6. Borneol
7. Camphor
8. Carene delta-3
9. Citral
10. Citronella oil (India Hydbd)
11. Citronella oil (SHK)
12. Citronella oil (VN)
13. Citronellal
14. Citronellol
15. Citronellyl acetate
16. Citronellyl butyrate
17. Citronellyl formate
18. Coriander oil
19. Cymbopogon jwarancusa oil
20. Eucalyptus oil (globulus SHK)
21. Eucalyptus hybrid oil
22. Geranium oil (Hybd.)
23. Geranium oil (SHK)
24. Geraniol
25. Geranyl acetate
26. Geranyl butyrate
27. Geranyl caproate
28. Geranyl formate
29. Ginger oil
30. Ionone Alpha
31. Ionone Beta
32. Isobornyl acetate
33. Jamrosa oil
34. Litsea cubeba oil (Vietnam)
35. Lemon grass oil (CKP-25)
36. Lemon grass oil (LS-48)
37. Lemon grass oil (SHK)
38. Linalool
39. Linalyl acetate

40. Mentha arvensis oil (Shivalik)
41. Mentha hybrid (Russian)
42. Mentha piperita
43. Mentha spicata
44. Mosmi 20 fold (citrus)
45. Palmarosa oil
46. Petitgrain oil
47. Pine oil (special)
48. Pinene Alpha
49. Pinene Beta
50. Terpeneol Alpha
51. Terpenyl acetate
52. Turpentine oil

COLUMN SE-30:

53. Agar Ruh
54. Anethole extra
55. Anisaldehyde
56. Anise oil (NBPGR)
57. Anise oil (Dabur)
58. Borneol
59. Camphor
60. Cinnamic alcohol
61. Cinnamic aldehyde
62. Cinnamon cassia oil (Vietnam)
63. Cinnamon leaf oil (SHK)
64. Cinnamon leaf oil (DABUR)
65. Cinnamyl acetate
66. Citronellal
67. Clocimum oil
68. Fokeinna hodginsii (VT)
69. Hydroxy citronellal
70. Iso eugenol
71. Iso bornyl acetate
72. Limonene
73. Ocimum basilicum oil (Egypt)
74. O. basilicum oil (Madagaskar)
75. O. basilicum oil (Indian cultivated)
76. O. basilicum oil (methyl chavicol type EC 291415)
77. O. basilicum (0095-Methyl cinnamate type)

78. O. basilicum(EC 174526 Linalool type)
79. O. basilicum(EC 282721 Eugenol, methyl chavicol type)
80. O.gratissimum (VN)
81. Patchouli oil (Indonesian-NBPGR)
82. Patchouli oil (SHK)
83. Sandal wood oil (SHK)
84. Terpinene Alpha
85. Terpineol Alpha
86. Terpenene-1-ol-4
87. Terpenyl acetate
88. Vetiver oil (Indonesian)
89. Vetiver oil (NC 66403, NBPGR)
90. Vetiver oil (South Indian)
91. Vetiverol
92. Vetiveryl acetate

ANNEXURE 11

LIST OF CAPALLARY GC'S OF VIETNAMESE OILS BROUGHT BY EXPERT

1. Cajeput oil.
2. *Cinnamomum cassia* oil.
3. Citronella oil.
4. *Fokeinna hodginsii* oil.
5. *Litsea cubeba* oil.
6. *Melaleuca leucadendron* oil.
7. *Ocimum gratissimum* oil.

ANNEXURE 12

OPERATION OF SHIMADZU GAS CHROMATOGRAPH

I. OPEN THE CARRIER GAS FROM CYLINDER (He/N_2):

1. Open the stopcock of the carrier gas.
2. Open the stopvalve on the right side of the GC main equipment.
3. Select the flow rate for column 1 or 2.

II. OPEN THE EQUIPMENT:

1. Turn on Aptomat Main Switch.
2. Turn on voltage stabilizer switch.
3. Turn on Power switch on GC-9A.
4. Put detector switch on with the help of Keys on main equipment.
5. Set the analysis program.
6. Turn on HEATER Switch on GC-9A.
7. Press 'START' button on GC-9A.
8. Turn on POWER on CR6A and Expansion Case.
9. Set the processing peak parameters at CR6A.

III. SUPPLY HYDROGEN GAS AND AIR:

1. Turn on Power Switch of Voltage Stabilizer for the air compressor.
2. Open the Stop valve of the air compressor.
3. Open the Stopcock of the Hydrogren Cylinder.
4. Open the Stop valve of Hydrogen Gas on the right side of the GC main equipment.
5. Select the Flow rate for column 1 or 2.
6. Ignition:
   - decrease air pressure : 0,1 # 0,2 kg/cm^2
   - increase Hydrogen pressure : 0,9 kg/cm^2
   - ignite
   - reset air pressure and Hydrogen pressure on the regular valve (Air 0.5; Hydrogen 0.6 kg/cm^2)
   - checking ignition by bringing glass slide to the detector top

IV. INJECTION OF SAMPLES:

1. Check two 'READY' lamps on both GC-9A and CR-6A.
2. Inject the samples.
3. Press two 'START' buttons on both GC-9A and CR-6A.

SETTING OF ANALYSIS PROGRAM ON GC-9A

1. Column oven temp. program:

   - Initial temperature: Ex. 60°C

     COL / AUX.1 → / I. TEMP. / → / 6 / → / 0 / → / ENT /

   - Time of retaining initial temp. Ex. 15 min

     COL / AUX.1 → / I. TIME / → / 1 / → / 5 / → / ENT /

   - Temp. program rate : Ex. 7°C/mm

     COL / AUX. 1 → / P. RATE / → / 7 / → / ENT /

   - Final temp. of column : Ex. 180°C

     COL / AUX. 1 → / F. TEMP. / → / 1 / → / 8 / → / 0 / → / ENT /

   - Time of retaining final temp.: Ex. 30 min

     COL / AUX.1 → / F. TIME / → / 3 / → / 0 / → / ENT /

2. INJECTOR TEMP.: Ex. 250°C

   INJ / AUX. 2 → / 2 / → / 5 / → / 0 / → / ENT /

3. DETECTOR: (FID)

   - Polarity Ex. 1

     / POL / → / 1 / → / ENT /

   - Range: 10^4

     / RANG / → / 4 / → / ENT /

MONITORING OF ANALYSIS PROGRAM : ON GC-9A

---

1. **TEMP. MONITOR :** + column temp.

/ MONI /

COL

AUX. 1

+ Inj. temp.

/ MONI /

INJ

AUX.2

2. **RETENTION TIME:**

/MONI/ /SHIFT.D/

STOP-W

RET.T

CONFIRMATION OF THE SET PROGRAM : ON GC-9A

1. COLUMN OVEN TEMP. PROGRAM:

- Initial temperature program :

COL
AUX.1

/ I. TEMP./ / ENT /

- Time of retaining initial temp.

COL
AUX.1

/ I. TIME / / ENT /

- Temp. program rate :

COL
AUX. 1

/ P. RATE / / ENT /

- Final temp.:

COL
AUX. 1

/ F. TEMP. / / ENT /

- Time of retaining final temp.:

COL
AUX.1

/ F. TIME / / ENT /

2. INJECTOR TEMP.:

INJ
AUX. 2

/ ENT /

SETTING OF PEAK PROCESSING PARAMETERS ON CR6A. PRINTER

/ WIDTH / / 5 / / ENTER /

/ SLOPE / / 2 / / 5 / / ENTER /

/ DRIFT / / 0 / / ENTER /

/ MIN AREA / / 1 / / 0 / / ENTER /

/ T. DBL / / 0 / / ENTER /

/ STOP. TM / / 6 / / 0 / / ENTER /

/ ATTEN / / 3 / / ENTER /

/ SPEED / / 4 / / ENTER /

/ SPL. WT / / 1 / / 0 / / 0 / / ENTER /

/ IS. WT / / 1 / / ENTER /

/ METHOD / / 4 / / 1 / / ENTER /

/ FORMAT / / 0 / / ENTER /

LEARNING OF THE SET PARAMETERS

/ S/D / / LIST / / WIDTH / / ENTER /

SIMPLE ANALYSIS OPERATION

a. / PRINT / / CTRL / / LEVEL / / ENTER /

b. / ZERO / / ENTER /

c. / S/D / / S. TEST / / ENTER /

d. Inject a sample.

e. / START /

SETTING OF CHROMATOPAC DISK BASE CR6A

PROCEDURE TO START UP:

1. Turn on the power of the CR6A, 6-slot expansion case, FDD-1A and CRT display.

2. "FDD-1A boot V1-0" is printed out.
   Set the system disk into the drive 1 of FDD-1A.

3. "FDD-1A FDOS V1.2" is printed out.
   Press the keys CTRL MOUNT ENTER

4. "CHROMATOPAC CR3A (DISK BASE) V" is printed out (displayed) after few minutes.

ANNEXURE 13

LIST OF GAS CHROMATOGRAMS OF ESSENTIAL OILS AND ISOLATES CARRIED OUT AT VINAROM

ESSENTIAL OILS:

01. Aloe wood (agarwood) oil (3-fractions)
02. Anise oil (Star)
03. Anise oil (India)
04. Cajeput oil
05. Cinnamon bark oil (Cassia)
06. Cedarwood oil
07. Citronella oil
08. Eucalyptus citriodora oil
09. Fokeinna hodginsii oil
10. Lemongrass oil
11. Litsea cubeba oil
12. L. cubeba oil (Enteroil)
13. Mentha arvensis oil (India)
14. Mentha arvensis oil
15. Ocimum basilicum (VN)
16. O. basilicum (Egypt)
17. O. basilicum (Madagaskar)
18. O. basilicum (0095, NBPGR, India)
19. O. gratissimum (VN)
20. Orange oil
21. Palmarosa oil (India)
22. Palmarosa oil (VN)
23. Pine oil (India)
24. Sandal wood oil
25. Sassafras oil
26. Turpentine oil (India)
27. Turpentine oil (VN)
28. Vetiver oil (SHK)
29. Vetiver oil (South India)
30. Vetiver oil (Indonesia)

ISOLATES:

31. Aldehyde C-10
32. Anisaldehyde
33. Benzaldehyde
34. Borneol
35. Camphor
36. l-Carvone
37. Caryophyllene
38. Cineol (1:8)
39. Cinnamaldehyde
40. Cinnamyl acetate
41. Cinnamyl alcohol
42. Citronellol
43. Citronellyl acetate
44. Citronellyl butyrate
45. Citronellyl formate
46. Elemene-Beta
47. Eugenol
48. Eugenyl acetate
49. Geraniol
50. Geranyl acetate
51. Geranyl butyrate
52. Geranyl caproate
53. Geranyl formate
54. Geranyl valerate
55. Iso eugenol
56. Limonene
57. Linalool
58. Linalyl acetate
59. l-Menthol
60. Menthone/Isomenthone
61. Methyl eugenol
62. Nerol
63. Ocimene Beta
64. Piperitone
65. Pulegone
66. Safrole
67. Terpene-1-ene-4-ol
68. Terpineol Alpha
69. Terpenyl acetate
70. Vanillin
71. Vetiverol
72. Vetiveryl acetate

ANNEXURE 14

TEST FOR GAS CHROMATOGRAPHY TRAINING

1. Write main principle of Gas Liquid Chromatography.

2. What is the most essential precaution you would take, while starting and closing G.C. equipment?

3. What is proof of successful injection?

4. Describe stationary phase and solid support.

5. Name carrier gases.

6. Name G.C. columns used for analysis of aroma chemicals.

7. Name different detectors used in G.C.

8. Write few words about Retention time.

9. What is peak area, how do you calculate it?

10. Differentiate between packed and capillary columns. What is advantage of using capillary columns?

11. How does Flame Ionization Dectector work?

12. Indicate maximum temperature for Carbowax 20M and SE-30 columns.

13. What is the criteria for choice of internal standard?

14. Comment on preparative G.C.

15. What is Response factor?

16. Which solvent often used for preparing slurry of stationery phase and support material for column packing?

17. What temperature do you use for conditioning of columns?

18. Which are more polar compounds amongst following pairs:
    (i) Phenols - alcohols
    (ii) citronellol - citronellylacetate
    (iii) Geraniol - geranyl butyrate
    (iv) Limonene - 1:8 cineole
    (v) Methyl chavicol - eugenol
    (vi) Geraniol - Geranyl formate
    (vii) C-10 aldehyde - C-10 alcohol
    (viii) Menthol - menthone
    (ix) Fatty alcohols - Fatty acids
    (x) Cis-anethole - trans - anethole

19. Name suitable colums to be used for G.C. analysis of following:

(i) Star anise oil	(ii) Litsea cubeba oil
(iii) Terpeneol isomers	(iv) Cassia oil
(v) Eugenol	(vi) Methyl chavicol
(vii) Mentha oils	(viii) Terpenyl acetate
(ix) Palmarosa oil	(x) Citronellyl acetate and citronellol

20. What G.C. parameters and column willyou use for analysis of turpentine oil? Please draw chromatogram.

ANNEXURE 15

BOOKS SUGGESTED FOR GAS CHROMATOGRAPHY

1. Basic Gas Chromatography by H. M. Mc. Nair and E. J. Bonelli, Pub. - Varian.

2. Chromatography by Erich Heftmann - Part B Applications - Pub.: -Elsevier.

3. Modern Practice of Gas Chromatography by R. L. Grob Pub : John Wiley & Sons.

4. Quantitative Analysis by Gas Chromatography by J. Novak. Pub. : Marcel Dekker.

5. Gas Chromatography Data Compil[illegible] by ASTM AMD 25-A-S1.

6. Essential Oil Analysis by capillary Gas Chromatography and Carbon - 13 NMR Spectroscopy by V. Formacek and K. H. Kubeczka, J. Wiley & Sons, New York (1982).

7. Analysis of Essential Oils by Gas Chromatography and Mass spectrometry by Yoshiro Masada: Pub. : Halsted Press Book, J. Wiley & Sons, Inc., New York (1976).

Backstopping Officer's Technical Comments based on the report of Mr. M. Maheshwari, Gas Chromatograph Expert DP/VIE/86/033/11-54

The report contains very useful information and technical details about the use of Gas Chromatographic techniques in essential oil analysis and the procedures for the synthesis of some aroma chemicals.

The duties listed in his job description have been fully realized and additional work as requested by the CTA has also been done. The expert has brought 110 reference samples and other valuable documents including 92 reference chromatograms which have not only helped in performing his task efficiently but also strengthened the reference facilities of the Institute for future work.

Backstopping Officer agrees with the recommendations of the expert to improve the efficiency of GC analysis and bench scale synthesis and hopes that the CTA and NPD will initiate action to requisition some of the items from available funds.