OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

17771

DP/ID/SER.A/1258
2 October 1989
Original: ENGLISH

DEVELOPMENT OF INDUSTRIAL PRODUCTION OF ESSENTIAL OILS, AROMAS AND FLAVOURS

DP/VIE/84/010

THE SOCIALIST REPUBLIC OF VIETNAM

Technical report: Processing of Vietnamese Essential Oil and Related Natural Products, Quality Control of Essential Oils*

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

Based on the work of Dr. A.L. Jayewardene
Industrial Chemist

Backstopping Officer: Dr. R.O.B. Wijesekera, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

* This document has not been edited.

V.89-59926

CONTENTS

- - - - - - -

ANNEXES

- - - - - - - -

ABBREVIATION

CNRS - Centre National du Recherohen Scientifique

(NCSR) - (National Centre for Scientific Research)

ENTEROIL - Essential Oils Enterprise in Vietnam

GLC, glc - Gas Liquid Chromatography

FID _ Flame ronisation Detector

T C D - Thermal Conductivity Detector

T L C - Thin Layer Chromatography

CARBOWAS - Polyethylene glycol was

SE 30 - Methyl Silicone polymev gum

D C M S _ Dicholro methyl silane

HMDZ - Hexamethyl disilayane

HPLC - High Performance Liquid Chromatography

IR - Infra Red

UV - Ultra Violet

2. INTRODUCTION

In October 1987, a Project Document was signed by the Government of the Socialist Republic of Vietnam and the United Nations Development Programme with the effective starting date as April, 1988.

The Project with a proposed duration 24 months bore the number DP/VIE/84/010/A/014.

The Government implementing authority was to be the NCSR (National Centre for Scientific Research) and the executing Agency for UNDP ws the United Nations Industrial Development Organisation (UNIDO).

The Development objectives of the Project was in line with the National Development goals as projected in the Five Year Plan for the period 1986-1990 and will contribute to the increase in production of Vietnamese essential oils and related natural products, thus enhancing rural development and providing raw materials for local industries not only ensuring their controlled development but, contributing also to earning of foreign exchange.

The immediate objectives of the Project was the building up of technical expertise and the developing of marketing links with overseas consumers. To achieve these goals the NCSR was appointed the principle organisation responsible for quality control and transfer of technology in the programme of increasing the production and improving the quality of Vietnamese essential oils and related products in order to contribute more effectively to the production of export oriented goods.

Furthermore, the coordinating activities of production and marketing have been centralized by establishing within the NCSR of ENTEROIL the Essential Oils Enterprise.

The UNDP inputs to the Project purchasing of selected instrumentation for quality control and equipment for Pilot Plant processing together with missions by agreed international experts. An Industrial Chemist with upto date experience in production and quality control of essential oils and related products using the latest instrumentation was to carry out a staff training mission.

The importance of an assurance of quality in the products to be exported to foreign consumers has been recognized and the training provided by this mission was to strengthen the efforts of the staff in the quality control laboratory established under the Project.

3. SUMMARY

The Consultant Industrial Chemist after a briefing by the CTA, Dr. Atal conducted a training programme for the staff of the quality control laboratory of ENTEROIL during an eight weeks period from 12. April to 2. June, 1989.

The instrumentation needed for quality assessment of essential oils had been installed in the laboratory. The Shimadzu gas liquid chromatograph was commissioned and quality control work was being carried out on essential oils. The work program was prepared (Annex) in consultation with the Chief Technical Adviser. It was decided to begin the program by providing some basic theoretical knowledge as background to each of the instrumental methods. A series of discussions-lectures were conducted based on the more important methods namely GLC, HPLC, IR, UV and TLC. Applications of each method

in analysis and interpretation of data were illustrated and discussed. A joint seminar on quality control and marketing was conducted with the Marketing Specialist. At this forum the need of quality control and how it was to be carried out were discussed. The importance of good quality in the successful marketing of goods was also highlighted.

The general discussions on basic theory were followed by demonstration using actual practial example of quality assessment. These were already being carried ut by the staff on specimens brought in by producers.

The main essential oils being analysed presently in the quality control laboratory are :

1. Citronella (Java) oil Cymbopogon winterianus
2. Ocimum basilicum oil
3. Ocimum gratissimum oil
4. Mentha arvensis oil

Among the other oils which have been analysed using the instrumental methods were :

Casssia oil - Cinnamonum Cassia Blume : -

Litsea cubeba oil : -

Pemou oil - Fokiena hodginsii : -

Cajaput oil - Melaleuca leucadendron : -

Star anise oil - Illicum verum : -

Homalomena aromatic oil

Though certain other essential oils have been produced in Vietnam in the past, no specimens of the following oils were received for analysis. Ylang Ylang (Canaga odorata), Palma rosa (Cymbopogon martini and Vetiver (Vetiveria zizanoides).

The following techniques were demonstrated and the officers obtained hands on experience in performing the same operations.

i. Preparation of TLC plates and the application of TLC in analysis

ii. Preparative TLC in isolating pure substances

iii. CLC analysis of a large number of essential oils and standard reference components

iv. Interpretation of data obtained from the computing intergrator.

v. Using the quantitative data in preparing quality assessment certificates

vi. Application of Physico-chemical methods in quality assessment of essential oils

vii. Sensory evaluation of specimens of essential oils using paper strips and judging of rates of evaluation, tenacity etc.

Instructions on drafting of suitable standards specifications for essential oils using the analytical data obtained over a time period were provided. Examples of such tentative standards were drawn up. The final goal of the exercise would be to establish Vietnam National Standards for the more important essential oils.

4. TRAINING OF THE STAFF IN THE QUALITY CONTROL LABORATORY

4.1 Overview

The three members of staff are :

Mr. Do Pho, Chief Technical Engineer

Mr. Mai Thanh Son, Electronics Engineer

Mr. Nyugen Quoc Dung, Chemist

These three officers were already performing quality assessment work on essential oils using the GLC equipment when the training

program was started. Mr. Son and Mr. Dung had been working with GLC equipment during their service at CNRS. They were competent and had a fair amount of practical knowledge. The Simadzu GC-9A with a computerised input keyboard and the C-R 6A computing intergrator were manipulated quite competently and they were getting results which were adequate for the quality control purposes in essential oils. Mr. Son also had practical experience in packing GLC columns and preparing them for analysis by conditioning over a time period. The basic knowledge and theory behind the various methods of instrumental analysis was lacking. In particular, the problems of unever response of detectors to different compounds was not realised and that the results from peak area determination of percentage composition could be somewhat erroneous. They had no knowledge or apprecation of the theory nor practical application regarding the other instrumental technique such as HPLC and Infra Red Spectrophotometry. The refractometer and Polarimeter had been used for some measurement on essential oils. The thin layer chromatography equipment had not been used for any analytical work though the officers knew that silica gel plates could be prepared by spreading a slurry on a glass plate by hand, they had not used the applicator previously.

4.2 Discussions and Demonstrations

The task of the training program was to provide the necessary background knowledge in GLC as well as in the other analytical technique for which instrumentation was installed.

Accordingly the following lecture discussions connected with the four main analytical instruments were conducted:

1. Basic gas chromatography theory and some application - Annex 6
2. Infra Red Spectrophotometry theory in outline and applications in organic chemical analysis
3. UV/Visible Spectrophotometry outline of theory and applications
4. High Performance Liquid Chromatography its theory and applications

4.2.1 Thin Layer Chromatography

The methodology of thin layer chromatography and its application in separation of essential oil component were demonstrated (Annex 8).

The principles of preparing suitable solvent systems and selecting of reagents for visualising were explained and practical demonstration made. The methods of preparative TLC for isolating small amounts of pure components was shown. Anethole of high purity was separated from star anise oil.

4.2.2 Theory and Application of Gas Liquid Chromatography

The basic introduction to gas chromatography was conducted as a discussion on the principles underlying chromatography and how these principles were used to separate volatile substances in a gaseous or vapour state. The great advantge GLC had over other analytical methods for analysis of essential oil was based on the relatively high volatality of these products and this was clearly explained and then the numerous applications besides analysis of essential oils were also explained.

A copy of the booklet titled an Introduction to Gas Chromatography - published by Pye Unichem and Phillips was handed over to the staff. The book published by Varian AG titled Basic Gas Chromatography was also available in the laboratory.

Application of GLC to the analysis of essential oils was discussed. It was pointed out that use in quality assessment was only one of a large number of possible applications as GLC. The methods used for identification of separated components were explained including the use of spectropic techniques to confirm the identification.

Quality assessment being the primary function of the ENTEROIL laboratory the following aspects were discussed and application of methods to actual specimens were demonstrated:

Sampling methods

Preparation of sample

Analysis on a suitable packed column

Collection of data on the computing intergrator

Interpretation of the raw data and application of the results to arrive at the quality of the specimen analysed

The three officers were already carrying out this part of the quality assessment work competently. They appreciated the background information the Consultant ws able to provide and they also get an understanding of the shortcomings of the method they were using.

The need for calibration of the detector response was strongly emphasised and the technique reported by the Consultant in the UNIDO Manual of Essential Oil Industry was explained in detail. The method where quantitative GLC is performed with an internal standard and reference standard mixture was demonstrated using examples.

Finally the use of this calibration results to adjust the percentage compositions obtained from the computer printouts was explained. (Annex 9).

Other aspects of glc discussed were the need for high purity of gases particularly the carrier gas which must not contain oxygen above trace level. The results of high oxygen levels was observable in columns, which deteriorated rather more quickly than usual. This matter is further discussed under observation in this report. The preparation of liquid phase coated packing materials was described. As solid support and stationary liquid phases were not available a demonstration was not possible. The basic guidelines and some hints not generally reported in literature were written out for the laboratory. (Annex 12)

A library of standard reference compounds ws established. The CTA was able to procure a number of reference compounds and standard essential oils from India. The samples were catalogued and a list prepared with best storage conditions for long shelf life (Annex 16).

4.2.3 Interpretation of data

The availability of a computing intergrator has made data acquisition and processing extremely simple. The computer printout includes a chart of the gas chromatogram as well as parameters such as peak number, retention time peak area and percentage composition based on peak areas. A number of other facilities are available in this machine such as standardisation with internal standard where the data manupulation could be done in the integrator itself.

4.2.4 Theory and practice of high performance liquid chromatography (HPLC)

The principles of HPLC were clearly explained based on :

i. Reduction in particle size of packing

ii. Attainment of very rapid equilibrium

iii. Application of very high pressure to increase solvent flow rate to accommodate the fast equilibrium

iv. Reduction of column dimensions and sample size

v. Availability of high sensitivity detectors using Refractive index UV/VIS Spectrophotometric and UV/fluoremetric detectors

The erros due to use of peak area data to obtain quantitative results because of widely varying response of detectors to different molecules and the importance of high purity solvents, and carefully prepared specimens were discussed. The care needed to prevent damage to the columns which are very costly by use of small guard columns was explained. The wide range of application in analysing quantitatively and qualitatively was discussed. Applications in analysis of essential oils were carried out. HPLC grade solvents other than Methanol were not available for more detailed work. Some methods for preparing high purity solvents from technical grade using the fractionation column and chemical treatment were suggested.

4.2.5 Methods of Chemical Analysis

The methodology for chemical analysis was discussed. It was pointed out that these methods were no longer widely used in QC of essential oils in international trade. They have been superceeded by gas chromatographic methods since of a decade. However standard specifications refer to these methods and were therefore thought to be relevant. On a number of occasions the determination of total phenols by chemical method was carried out and was found to be satisfactory for quality assurance when performed with great care. The methods for performing these chemical analysis are described in the specifications of all standards organisations, and in the UNIDO manual of Essential Oil Industry.

4.2.6 Other Methods of Instrumental Analysis

The theory and applications of Infra Red (IR) and Ultra Violet (UV) spectrophotometry were explained. The IR instrument was out of order and all attempts to put it back in order were of no avail. The operating manual gives some hints on tracing faults none of which were of any use. Consequently no specimens could be analysed in order to obtain IR spectra. The method of identifying organic substances with the help of IR spectra wcs explained and the manner in which the finger print region (12 - 18 microns) was used to authenticate the simpler organic molecules ws also dexcribed.

The UV spectrophotometer is a single beam instrument and it had been supplied without a printer or a recorder. If the printer is obtained it would be useful for determining the concentrations of single substances which have absorption bands in the UV/Visible?NIR range. The method of making a manual plot was described and also the use of calibration standads for obtaining true concentrations are found in the operators manual.

4.2.7 Standard Specifications for Essential ' ls

The establishment of standards specifications for the main essential oils produced in the country was considered useful because:

i. The different grades of each type of oil could then be accommodated within such standards.

ii. These specifications culd be formulated to fit within the minimum requirements of the International standards since the composition of the oils are within such limits.

iii. The data already collected in the laboratory from the analysis of numerous commercial samples would be helpful in determining appropriate limits.

iv. Such a set of standards for the main oils would be of advantage for ENTEROIL as their oils could then be presented to the buyers backed by specifications of physico-chemical characteristics.

v. These tentative specifications could also reflect the GLC analytical data.

4.2.8 Laboratory scale extraction of essential oils

ISO Standards modified Clevenger Apparatus ws assembled in the laboratory and a number of specimens of Ocimum gratissimum one of O.basilicum (French), and one specimen each of Star Anise and citronella java were distilled and the oils collected. The value of such distillations for experimentally monitoring conditions during field distillations was shown. In a trial conducted in a citronella growing region it was possible to show that the quality of the grass that was fed to the still was quite good contrary to the belief held by the producer.

4.2.9 Other Activities

Visits : The consultant accompanied by the Marketing Consultant visited a farm in Hai Hung province where mainly Mentha arvensis and O. gratissimum was being grown and their oils were being produced.

Some suggestions on the proper storage and packaging of essential oil were discussed and a report submitted (Annex). The need for a more appropriate sample bottles in two sizes of 25ml and 50ml preferably of aluminium was stressed.

Discussion: The CTA of the Project VIE/87/015 on the Duc-Giang Pure Chemicals Factory and a Consultant had discussions regarding instrumentation for analysis, installation and commissioning and problems of supplies.

5. CONCLUSIONS

The training programme on quality control of essential oil conducted for the staff of the laboratory on quality control at ENTEROIL achieved the following objectives.

1. The three officers were given a good background on the theory and a clear understanding of the applications of GLC, HPLC, IR and UV spectrophotometry in the quality assessment process.

2. The officers also gained knowledge on the care, maintenance and servicing of sophisticated analytical instruments installed in the laboratory.

3. The need for calibration of the detectors to compensate for variable response in both the GLC and HPLC was stressed and a method of calibration using reference standards with an internal standard and a sample with the same internal standard was demonstrated. The greater reliability of quantitative results after such calibration was illustrated.

4. A library of standard reference compounds and essential oils was established. The set of specimens obtained through project funds was catalogued and the most suitable storage conditions and locations indicated for ease of access.

5. The need for high purity carier gas for the GLC was stressed. The inability to operate the GLC at higher sensitivities due to this reason were pointed out. A case for supplying the lab with Nitrogen of 5-nines (99.999%) purity or pure Argon as an alternative.

6. The need to protect the high cost HPLC analytical columns by incorporating a guard column immediately ahead of the main analytical column was pointed out and also the necessity of very high purity solvents which have been filtered through very fine membrane filter.

7. The application of the thin layer spreader to prepare hand costed TLC plates was demonstrated.

8. The preparation of authentic specimens of essential oils from correctly identified raw materials in the laboratory using the Clevenger apparatus was demonstrated.

9. A method for refining strongly coloured phenolic oils in order to reduce the colour using chemical treatment was described and decolourised specimens prepared.

10. Information on the correct methods of storage an packaging of essential oils was provided. Suggestions for suitable containers for storage and shipment of oils, were made.

6. RECOMMENDATIONS

1. To obtain the full use of the Gas Chromatograph high purity Nitrogen (99.999% or better) of guaranteed quality should be obtained. If unavailable in the country a few cylinders should be shipped in from a neighbouring country.

2. As the temporary measure a gas purifier could be attached to the Nitrogen line immediately after the exit from the regulator. Annex No. 17 give details of such purifiers.

3. A list of recommended spares, accessories and standards etc. is annexed. It would be desirable to have these materials supplied to the laboratory in order that full use of the instruments is achieved in time to come.

4. Though methods for producing high purity solvents from technical grades have been discussed it would be more preferable to provide the laboratory with the small quantities of HPLC grade solvents as listed in the annexe.

5. A single pen 1mv Potentiometric recorder would be an asset to the lab as it could be coupled to either the GLC or the HPLC during periods of breakdown of either C-R6A Computer integrators. Furthermore the cost of operating the chart recorder is appreciably lower than using the integrator mainly because the special thermal paper it uses is more costly. IN analysis where peak area integration and other data is not essential and only the patterns are important a chart recorder is quite sufficient. The thin strip chart paper facilitates examination of master charts with sample charts by super posing one over the other.

6. The packing of GLC columns is both economical and of advantage because special mixed liquid phase packing could be prepared for packing. The standard packing materials could be purchased or the materials for making the packing could be procured seperately both material requirements are listed.

7. The printer for the UV/VIS/NIR Spectrophotometer should be purchased.

8. A dehumidifier for the instrument room is urgently required. During the period middle of May to October the relative humidity is extremely high and the highly sophisticated electronics can easily malfunction under such conditions.

9. The infrared spectrophotometer should be put back in order as soon as possible. A telex requesting assistance from supplier to send an engineer in the region has been sent.

10. Further training in a practical course on applications of HPLC in analysis and quality assessment. The course should include training in maximising the use of the C-R6A computing integrator to get the widest possible use from this valuable instrument.

11. A training program in maintenance servicing and repair of electronic analytical instruments would be most beneficial to the laboratory in order to keep the instrument working throughout their life time.

ANNEX I

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

DP/VIE/84/010/11-02/J13422
Project in the Government of Vietnam

JOB DESCRIPTION

Post title	Industrial Chemist (Essential Oils)
Duration	2 m/m
Date required	
Duty station	Hanoi, with internal travel
Purpose of project	Processing of Vietnamese essential oils and related natural products
Duties	The expoert will function as part of a five-man team assisting the National Center for Scientific Research in the Socialist Republic of Vietnam in the development of its resources of materials of botanic origin. He will be responsible to the Chief Technical Adviser for carrying out demonstrations of up-to-date field hydro distillation techniques and recommending improvements where necessary. He will work closely with the appointed national counterpart. The expert will also be expected to prepare a final report, setting out the findings of his mission and his recommendations to the Government of further action which might be taken.
Qualifications:	A Chemist or Technologist with field experience in the distillation of plant materials for the production of essential oils. Must be knowledgeable in methods of instrumental analyses involving GLC.

Applications and communications regarding this Job Description should be sent to:

Project Personnel Recruitment Section, Industrial Operations Division
UNIDO VIENNA INTERNATIONAL CENTRE P.O. [illegible]

ANNEX 2

PERSONS AND INSTITUTIONS CONTRACTED

ENTEROIL

Mr. Le Van Thu	- General Director, Essential Oils Enterprise
Mr. Le Trong Vong	- Deputy General Director
Mr. Van Ngoc Dank	- Marketing Manager
Mr. Le Nhi Hoa	- Production Manager
Mr. Do Pho	- Chief Technical Engineer & Chief of Quality control
Mr. Nguyen Quoc Dung	- Quality control Labolatory Staff
Mr. Mai Thanh Son	- Quality control Labolatory Staff
Mr. Nguyen Nha Duc	- Assistant Marketing Manager Secretary Project DP/VIE/84/010

PROJECT DP/VIE/84/010

Dr. C.K. Atal	- Chief Technical Adviser
Mr. John G. Meredith	- Marketing Consultant
Mr. N.B. Narasimha	- Chemical Engineer Consultant

CNRS

Dr. Nguyen Quyet Chien	- Organic Chemist

UNDP/UNIDO

Mr. David Smith	- Resident Representative
Mr. T. Rose	- Deputy Resident Representative
Mr. Jean Marc Bonnamy	- SIDFA-UNIDO
Mr. Pham Duc Thang	- Programme Officer
Dr. R.O.B. Wijesekera	- Special Technical Adviser UNIDO VIENNA

VISITORS TO LABOLATORY

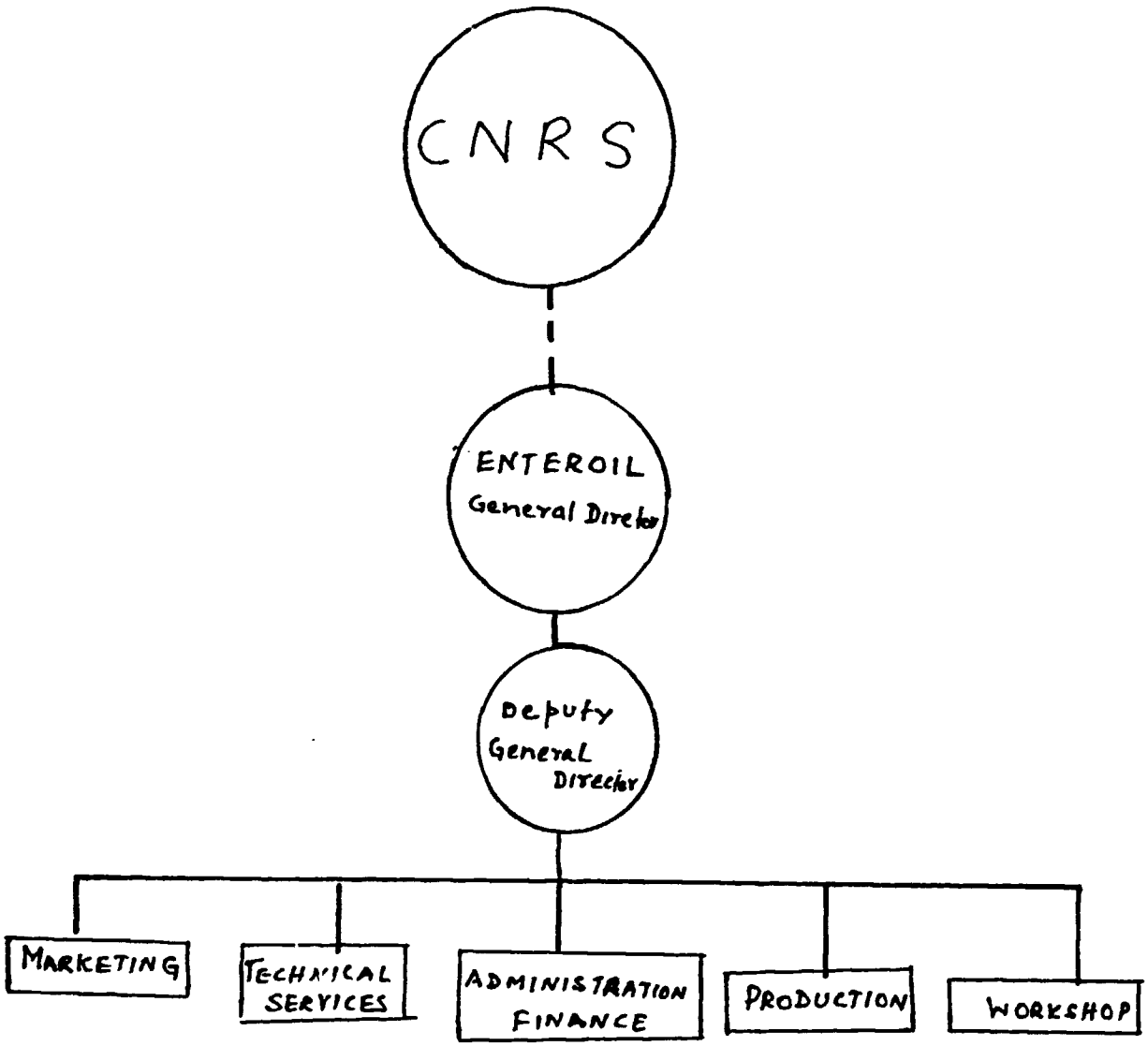
Dr. P. Rodzwadovsky	- CTA Project VIE/07/015, Duc giang Pure Chemical Factory
Dr. Zolamt Holmais	- Consultant
Mr. Dan de Mirmon	- Managing Director EUROPASIA CO Hong Kong

ANNEX 3

WORK PROGRAM - QUALITY CONTROL of ESSENTIAL OILS
12 April to 2 June 1989 A. L. JAYEWARDENE

1	WEEK OF APRIL 12 - 16	Arrive in Hanoi with Mr. John Meredith, marketing Consultant Meeting at ENTEROIL. Visit Quality Control Laboratory. Duscussions. Visit UNDP-Hanoi	
2	WEEK OF APRIL 17 - 23	Briefing by CTA, Dr. C.K. Atal Work program planned, Instructions and lecture discussions Introduction to Gas Chromatography. Theory and applications Technique of TLC analysis. Attend seminar on marketing	
3	WEEK OF APRIL 24 - 30	Methods of Quality control used in the essential Oil industry Physical methods, chemical methods appearance and Sensory evaluation. Seminar jointly with Mr. J. Meredith Visit to producing region. Joint seminar on Quality control and marketing	
4	WEEK OF MAY 1 - 7	Methods of preparative TLC. Isolation of pure compounds. UV and Ir spectrophoto metric Methods. Installation of large essential Oil still - in Pilot Plant. Carried out by Mr. MB Narasimha (Chemical Engineer) Consultant.	
5	WEEK OF MAY 8 - 14	Quantitative Methods of GLC analysis application to essential oils Visit by Dr. R.O.B. Wijesekera HPLC Analysis, Library of reference compund - set up	Tripartite meeting at Enteroil
6	WEEK OF MAY 15 - 21	GLC applications continued Internal Standard Method of Calibratives Use of Polarimeter and Refractometer for actual specimens - from producer	
7	WEEK OF MAY 22 - 28	Coated GL packing. Material preparation. Draw up tentative standards for oils. write out notes on procedures for giudance. complete draft of Report	Visit to Hà Tuyên province - Citronella still & Plantation
8	WEEK OF MAY 30 JUNE 4	Final discussions. Laboratory distillation of essential oils. Report final draft to CTA	
9	June 4	Departure for Vienna - debriefing	

ORGANISATION CHART
(Project VIE/84/010).



ANNEX 5

THE PURPOSE OF QUALITY CONTROL

The task in quality control of essential oils is to select the cheapest (most convenient) possible goal-oriented determination of the parameters which would allow producers to come up everytime with the right product having the right properties. These must also reveal if any adulteration and/or substitutions have taken place. Notwithstanding all this if the aroma or flavour are unsatisfactory (un-characteristic) then the purchaser would be still reluctant to buy the oil. As can be seen from the above the mission of quality control today is a complex and manifold one. The practitioner of quality control (the quality assessor) in turn has to have a good practical grounding in basic physico-chemical methods of testing and also some understanding of the applications of instrumental methods in quality assessment. Finally he must also have a reasonably sensitive and selective senses of smell and taste to perform the sensory part of quality evaluations.

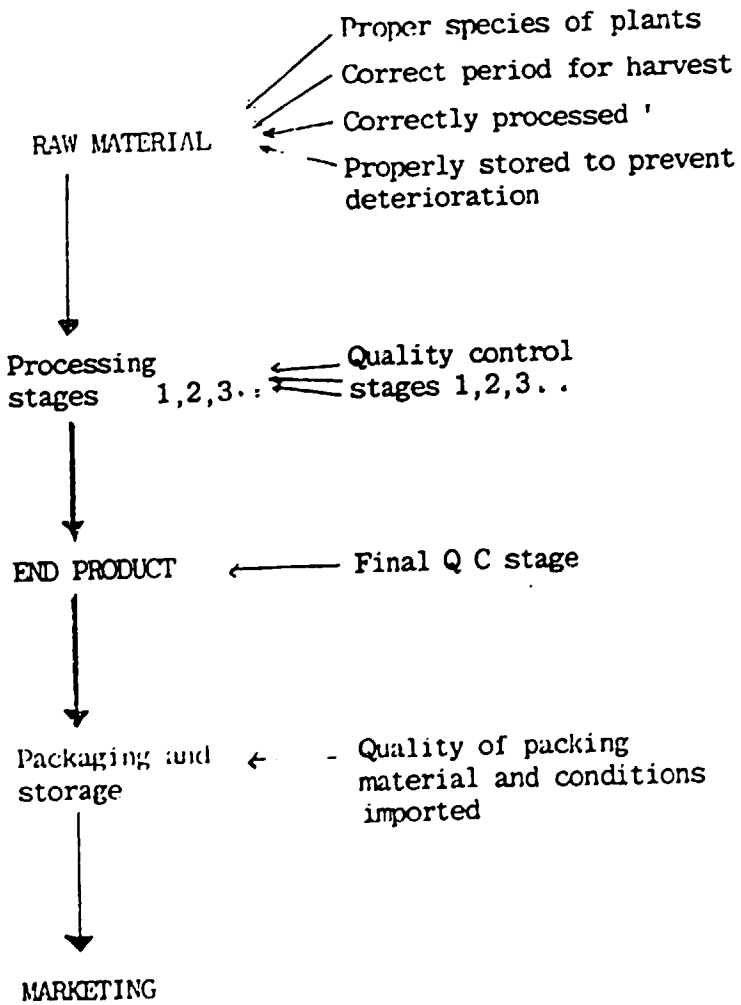
The recommended parameters mentioned before are compiled in sets of specifications. Many authorities both in producing countries (majority are developing countries) and consuming countries (mainly the developed West and Japan) issue specifications for essential oils. These may be either official standards or merely recommendations designed to assist quality maintenance and judgement. Among these the British Standards (BS), the Indian Standards (IS) the German DIN Standards, the French AFNOR Standards, the Essential Oils Association (EOA) U.S. Standards and the International Standards Organisation (ISO) specifications are the most important.

Many others including the British Pharmacopoeia (BP) the U.S. Pharmacopoeia (USP) and the European Pharmacopoeia can be mentioned. Most of these specifications contain the physical constants such as refractive index, specific gravity, optical rotation and specific rotation; non-volatile residue, solubility in ethyl alcohol (ethanol) of appropriate strength, relevant to each type of oil : The chemical parameters include the acid value, ester value the ester value after acetylation; (the difference of these two will give the value for free alcohol content), the carboxyl value (total aldehydes and ketones) and the phenol content. Many of the more important specifications include data on gas liquid chromatographic (GLC) analysis both qualitative and quantitative. However it must be emphasised that physico-chemical data alone cannot give more than a basic indication of the true quality of an essential oil, It is only by coupling this with the sensory evaluation that a complete overall picture of quality be obtained. For instance most freshly distilled oils answer all the quality tests flawlessly and the GLC analysis show a perfect specimen yet a trained nose will detect the unusual top-note quite commonly observed in freshly processed oils. It is only after a few week under good storage condition that the characteristic odour of the individual oil can be noted.

It is well known that quality assessment starts at the raw material stage in most processing industries. In the

essential oil industry too raw material of guaranteed quality is one sure way of obtaining quality assured final products. Since raw materials for essential oils give yield ranging from 0,5-6% by weight (exception being clove buds - 11-15%) there is corresponding increase in the value of final product. Therefore selection and grading of the starting material is well worth the extra time and money spent because, all other things remaining equal good raw materials give higher yields and superior quality essential oils.

THE QUALITY CONTROL STAGES



A N N E X 6

AN INTRODUCTION TO GAS CHROMATOGRAPHY

The following topics were covered :

1 . Theory of Gas liquid Chromatography.

2 . Instrumentation :

2.1 Columns.

2.2 Carrier gas.

2.3 Column selection and performance.

2.4 Capillary columns.

2.5 Column oven .

2.6 Isothermal and Programmed Temperature GLC .

2.7 Injectors.

2.8 Detectors.

2.9 Amplifiers.

2.10 Recorders and Integrators.

2.11 Accessories.

3. Chromatogram Interpretation.

3.1 Qualitative analysis.

3.2 Quantitative analysis.

4. Other topics of interest :

4.1 Preparation of columns.

4.2 Preparation of column Packing material.

4.3 Maintainance and service of instruments.

MAINTAINANCE AND SERVICE SCHEDULES (GLC)

1. Injection septa need periodic replacement.
2. Gas filtres and locular seive type purifiers need frequent checking and if found to be exhausted they should be re-ac_ivated or replaced depending on the type.
3. The flame ion:sation detector should be cleaned at regular interval. The flame tip should be examined and cleaned very carefully to avoid breadage.
4. The rota-meter gas flow rate indicators should be checked against the soap bubble flow meter.
5. It would be advisable to check the carrier gas flow at the detector end of the column.
6. The hydrogen generator should be regularly filled up with double distilled water. It is more preferable to use deionised water prepared from distilled water.
7. The microlitre syringes are precision instruments and they should be thoroughly cleaned after each injection preferable with acetone. Most damage to syringes occur as result of substnaces oxidising or polymegesing inside due to insufficient cleaning.

It is suggested that the syringe is flushed at least ten times with acetone filling it completely using the piston and then emplying each time into a waste bottle.

The well washed syringe may be occasionally cleaned more fully by filling with dilute chromic acid/sulphuric acid micture Potassium dichromate 5g in water 30 ml to which some of conc. sulphiric acid is added makes a suitable solution. After about hour the syringe is cleaned thoroughly with distilled water and then with acetone.

Packed glc columns should not be heated in the oven without carrier gas. The carrier gases usually nitrogen should be of high purity. It should not contain any oil vapour or traces of oxygen. The level of oxygen should be below 10 ppm if glc columns are to give any useful period of service.

In the absence of high purity nitrogen, argon gas used in manufacture of incandescent bulbs could be used satisfactorily.

USE OF REFERENCE GLC CHARTS (MASTER CHARTS) FOR INDENTIFICATION OF COMPONENTS IN ESSENTIAL OILS

The following steps are carried out:

(i) Prepare the master reference charts using

(a) A mixture of known-reference standards of high purity

(b)Pure essential oils of known chemical composition such as Citronella oil, Java Citronella oil Sri Lanka, Cassia oil, Ocimum basilicum oil, etc.

The reference samples are analysed on the glc under standardised conditions which included columns packing, liquid phase, lenght, flow rate of carrier gas, sensitivity of amplifiers and recorder chart speed.

Once the charts are obtained they can be clearly marked and the peaks identified by name. Reference to literature and published work is useful if previous knowledge is sufficient usually retention data is sufficient to identify components in a mixture of pure standards chemicals.

When the master reference charts are completed they can be used for identifying the probable chemical constituents of other essential oils or mixtures of chemicals or even fraction of oils obtained by distillation.

To do this the unknown samples are analysed under identifical conditions to those use to prepare the master charts.

Then by calculating retention times and relative retention times of samples chart they could be used to identify compounds with similar or exactly matching retention data. Sometimes, it is possible to superimpose the sample chart over the master chart and mark out the corresponding compound by name.

Such dientifications are always tentative even when data from two different GLC columns are used. To make the identifications more certain the components identified by master chart, comparison can be added in tiny amounts to small samples of the unknown oil and analysed by GLC if the identifiers are correct then the peak corresponding to the added substances will show up with higher response. If incorrect then some additional peak may appear where none existed in the chart of the pure sample.

OPERATION,CARE,MAINTAINANCE OF HPLC EQUIPMENT

1. Switch on and allow instrument to stabilize.
2. Switch on D2-Lamp and allow 20 min to stabilize.
3. Set required parameters of flow rate, detector wave lenght etc.....
4. Always use filtred degassed high purity solvents for HPLC
5. The change over procedure from solvent to another is given in the user manual.
6. Reinembev the reserve phase columns are run with high polarity solvents such as Menthanol, Methanol/water (70/30) or acetonitril and water 70:30. The latter is one of the most versatile solvents used in HPLC.
7. Remember that all solvents cannot be used with the UV-detector. Some solvent have strong UV absoprtion bands below 300 mm.
8. The UV-detector is extremely sensitive at low wavelenght and only very dilute solution should be injected as samples. Failing to observe this will result in a contaminated detector cell, which will give a high base line for long periods of time.

To prolong the life of HPLC columns please install " Guard columns " with the same packing as the column packing at the inlet end of the column.

ANNEX 8

PRACTISE OF THIN LAYER CHROMATOGRAPHY

1. PREPARATION OF THIN LAYER PLATES WITH SILICAGEL

1.1. Preparation of Silicagel G Slurry

1.2. Use of Manual Spreader to coat glass plate

1.3. Drying and activation in oven

1.4. Storage of plates in dry chamber

2. TLC ANALYSIS

1. Need of a template to mark out plate
2. Spotting samples-Template useful
3. Desion of solvent systems for different types of analysis
4. Development of spotted TLC plate in solvent tank
5. Application of spray reagent or Iodine UR VISU alise the separared Components
6. Marking out spots calculation of R f
7. Preparative TLC on thick layers
8. Visualise bands of components
9. Scraping out and Eluting the substance
10. Determine Purity
11. Use of pre-coafed TLC plates for semi-quantitative work with densitomess scanning

ANNEX 9

A Convenient Internal Standard Method for Quantitative Determination by Gas Chromatography

The technique described below has been used for quantitative analysis by glc of such diverse material as single aroma chemicals, essential oils petroleum hydrocarbons, pesticides, alcohols and alcoholic beverages, pharmaceutical and others.

METHOD

1. Prepare two solutions accurately in two volumetric flasks (a) A reference calibration mixture containing the pure compound(c) in need of quantitation together with an accurately known amount of suitable internal standards (b) A sample mixture containing a known weight of the sample in which the compound (c) is to be quantitatively measured together with an identical amount of internal standard as in (a). Both mixtures made up to the same volume with a suitable solvent.

2. Select the analytical parameters suitable for glc analysis of the substance in question and inject a suitable aliquot of both reference and sample mixture in triplicate to obtain 3 sets of data.

3. Calculation

Measure the peak areas of the compound (c) and internal standard (I_s) from the charts of the reference and the sample. Take the means of these value and compute the peak area ratios as follows

$$PA_{(R)} \quad \frac{\text{Peak area of (c) in reference}}{\text{Peak area of } (I_S) \text{ in reference}}$$

$$PA_{(S)} \quad \frac{\text{Peak area of (C) in sample}}{\text{Peak area of } (I_S) \text{ in sample}}$$

$$\text{Then the percentage of } (C)_{w/w} = \frac{PA_{(S)}}{PA_{(R)}} \times \frac{100}{m_S} \times M_R$$

where M_R and m_S are the mass of (c) in reference and mass of sample taken for preparation of the solutions.

PA_R is peak area ratio of reference mixture

$RA_{(S)}$ is peak area ratios of sample mixture

Example

Determination of eugenol in clove leaf oil

A. Reference standard :

Pure eugenol 0,50 g
Pure thymol (I_S) 0,10 g
Ethyl acetate to 100 ml

Sample mixture :

Clove leaf oil 0,60 g
Pure thymol 0.10 g
Ethyl acetate to 100 ml.

GLC parameters

Column and phase 2m long Carbowax 20M 10% on Celite
Column Temperature 185°C isothermal
Gas flow rate 25 ml/min
Detector and injector 225° and 200°C
Sample size 1.0 ul
Sensitivity 10^{00} x 64
Integrator if available.

Results

Mean peak area ratios were

Reference $PA_R = \frac{475}{118} = 4,00$

Sample $PA_{(s)} = \frac{398}{102} = 3,90$

$$\% \text{ w/w Eugenol in clove leaf oil} = \frac{PA_{(s)}}{PA_{(R)}} \times \frac{100}{0,50} \times 0,50$$

$$= 81,25$$

Advantages

The main advantage of this method is that the quantity injected need not be known accurately. Only the peak areas of the relevant peaks need to be measured while all irrelevant peaks are ignored.

Precautions

1. The internal standard must be selected carefully such that its peak does not over-lap any component peak in the specimen

2. It should have a retention distance close to that of the component of interest.

3. The reference compound and internal standard should be of the highest purity available.

A TYPICAL STANDARD SPECIFICATION FOR AN ESSENTIAL OIL

SPECIFICATION FOR OIL OF LEMON GRASS

Botanical Origin: - Cymbopogon Felxuosus (DC) Stap

General Name: - Oil if Lemon grass (East Indian)

Appearance: - Yellow to reddish-brown clear liquid with a strong lemon-like odour characteristic of oil of lemon grass

Physical parameter:

Specific Gravity: 0,895 - 0,910 (25^{o})

Optical Rotation: $-1^{o}10'$ to $-3^{o}10'$ (25^{o})

Refracture Index: 1,4847 - 1,889 (25^{o})

Solubility in Ethanol: in 2-2,5 volume or more of 70 % alcohol

Chemical Parameter:

Total Aldehyde Content: 75 % or more

Storage: Store in well closed containers filled up to neck or brim. A cool storage space in preferable tends to poly-nerise on long storage.

Containers: Shipping is preferable in Galvanised steeld drums or resin coated steel drums

Note: This specification is according to the essential oils association of America.

ANNEX 11

STORAGE AND PACKAGING OF ESSENTIAL OILS

One of the first impressions we get on examining a specimen of essential oil is the aroma of the material. This is made possible because of volatality of essential oils as explained in previous chapters. The first principle to be observed during the storage of essential oils is the prevention of loss by evaporation. Tightly closed vessels with well sealed stoppers are important for this purpose. The next observation we could easily make is the colour of the oil when a small amount is withdrawn from a metal container. In general all essential oils of good quality should be almost colourless (water white) pale yellow or pale brown in appearance. There are a few exceptions which may have very pale bluish or green tints. Essential oils should also be free of suspended matter, dust and they should appear clear and sparkling, when taken into a clear glass container.

The producer will do his very best to ensure maximum quality control during all processing stages and obtain superior quality oils. However if a mistake is made in storage and packaging all the good efforts put in to the production of the oil will be wasted.

Unfortunately there is no universally acceptable container for storage of essential oils. Different oils with various sensitive or reactive constituents need containers made of suitable material which will not react with constituents in oil, allow some components to escape or absorb them. Other consideration to be taken into account of are the size of each vessel the gross weight of the package and ease of handling safety in transport etc. Consider some of the common forms of containers we can obtain :

Glass bottles, jars and carboys of various sizes are available for packaging liquids. In many countries such containers are re-used after the initial contents in them have been removed. They come in colourless or pale green and amber shades, have fairly small necks which can be securely closed. All in all these containers appear to be ideal for storage and packaging of

essential oils. Two problems are inherent to all glass vessels, they are fragile and quite easily damaged. A twenty five litre consignment of some precious essential oil in a glass container breaking up due to some accident can be disastrous in every possible way. The monetary loss alone would be as high as 6000 dollars or more. The second problem is glass containers are heavy and if packaging for air freight is needed the cost for extra weight can be significant.

To avoid these problems cans and barrels made of various metals are now used by most oil producers. The metals that can be used with different types of oil have to be carefully selected. Generally iron or steel sheet, galvanized or tinned steel aluminium and stainless steel are the metals used for making containers. There are now various plastic resin coatings which can be sprayed on the inside of iron and steel drums for protection of essential oils from contact reaction with these metals.

Iron or steel drums and barrels are used for bulk storage and transport of non-phenolic essential oils such as citronella, lemon grass turpentine etc. With a good foolproof coating of a suitable plastic resin even these drums can be used for more valuable oils such as nutmeg, pepper and other spice oils. Even though phenolic oils are stable when stored in resin lined drums, there have been many instances where due to almost invisible faults in the resin coating the whole drums of clove oils and cinnamon leaf oil have been ruined. This is a well known reaction where the Eugenol found in large amounts in these oils attacks the iron to give inky black products. It has been found that the only way to purify such oil is to re-distill the material which results in a loss of upto twenty percent of the oil.

Aluminium is a fairly inert metal and well made containers are strong and fairly light in weight. Research studies have shown that aluminium containers are good for short term storage upto six month duration for most types of oil. Many kinds of oils and also perfumery and flavour materials are stored and transported in aluminium containers.

Stainless steel being quite expensive may be used for containers needed for storage of the most valuable oils which are suceptible to even aluminium. However stainless steel is much stronger than aluminium, therefore thinner sheet material can be used for containers.

Plastic containers.

Many plastic materials are quite unsuitable for storage and packaging of essential oil. Polyethylene though it is one of the most widely used plastic packaging material is totally unsuitable for storage or packaging of essential oils. In fact studies have shown the nearly all volatile aromatic substance are permeable through polyethylene barriers. Accordingly essential oils stored in such plastic material will quickly lose many of the volatile components resulting in deterioration of the quality of these oils, among the plastics a suitable material for essential oils is polyethylene terephthalate (PET) which has excellent barrier properties. However this material is quite costly. Some guidelines for storage of essential oils would be :

(a) Store oils only in containers which can be filled upto the brim. This prevents a headspace of air remaining in the vessel to cause oxidation of components.

(b) Select containers of the correct material as discussed above. Bulk oils can be stored in inexpensive steel drums provided they are free of moisture. Otherwise rust will form and contaminate the oil. Phenolic oils should never be stored in bare steel or galvanized drums. Use steel drums coated with double layer of plastic resin or aluminium drums.

(c) Glass containers if sufficient numbers and of fairly large size are available can be used for all essential oils to be stored.

(d) The more valuable oils are best kept in glass or stainless steel containers and shipped in the latter containers. However if the transit time is short aluminium may be accepted provided the purchaser transfers the oil to safer materials and/or uses the oil quickly.

(e) Stoppers used for closures should also be of resistant materials. Rubber and inferior quality plastic should never be used.

(f) Polythene should not be used for storage and packaging of essential oils.

There are certain precautions that can prove important for improving the appearance of oils and help to keep them safely during prolong storage. It will be observed that the oils as these separate out in the florentine vessels contain droplets of water, suspended matter and may appear cloudy. Furthermore freshly distilled oils will have certain off odours caused by other highly volatile material which distill over. These off odour disappear after a day or two and the real aroma of the oils can then be observed. The first step of treatment for the oil fresh from the distillation is to filter it through filter paper, cotton wool or in the case of bulky oil many producers use well washed lint free cloth bags usually made of white drill cloth. The advantage being they can be cleaned and reused. A double or even triple filtration can be recommended such that the final oil which goes into the dry, clean dust free containers are perfectly clear and sparkling in appearance, such specimens will keep in storage safely over long periods of time if the container are kept well filled and tightly closed.

Experience shows that essential oil keep longer and in better condition If they are stored at low temperature (10-15^{o}c).It is good practice to keep the filtered and dried oil in containers in a storaged rooms which is at least airconditioned and held at around 20^{o}c. This is particularly applicable to those oils which are more sensitive to normal conditions for example oil Cardamons,oil of Ginger,oil of Pepper and from flowers.

ANNEX 11

PACKAGING OF ESSENTIAL OILS

(Some recommendations)

The standardized bulk package for essential oils are 205 litre steel drums. The following protective coatings can be recommended:

1. Gavanizing
2. Tin plate steel
3. Epoxy resin coated and baked

The types of oil which could be stored in each of these types of drums have to be noted carefully to prevent contaminations.

SAMPLE VIALS

The ideal sample vials are 25 ML and 50 ml spun aluminium containers with white phenol-plastic cap with foil liner and a poly ethylene snap-in stopper. Some of the vials were provided by M.Dan de Mirmor, Gereral Manager, Europasia of Hingkong who visited Enteroil

ANNEX 12

SOME HINTS FOR OPERATION OF GLC INSTRUMENT

Starting up the various functional parts such as power, carrier gas switching on the amplifiers recorder and igriting the flame ionisation detector are procedures which are found in the manual given with each instrument.

In addition, there are certain other procedures which are followed by gas chromatorgraphers through experience gained over a period of operation of different instruments. Some of these steps are noted below.

1.1. On starting up the GLC system, it should be allowed to warm-up for 10-15 mins. Then with the correct carrier gas flow rate in the column, an initial temperature programme of the column oven is performed without introduction of any sample. This is called a blank programme and it helps to clear out (purge) any residual low rotatile material which maz have been left from the previous days operation even when the column is not changed. It is good practise to do a blank programme to prevent ghost peaks (i.e. peak which should not come out of the specimen) from being recorded in Chromatograms. Note that a blank programme and a fairly long hold at the final temperature is MANDATORY whenever one column is changed for another in the Chromatograph.

1.2. During isothermal g.l.c. analysis it is assumed that all injected substances elute out before the next injection. there can be however residual low volatile matter which will give rise to ghost peaks at a later time. The best way to avoid this problem is to purge the column at a higher temperature (10^{o}C below the column maximum) for 15-20 mins. This need to be done only after every ten or fifteen injections.

1.3. Column Clean-up.

This is most easily carried out by injecting a suitable volatile cleaning agent inot the packed columns which are first heated up to the highest operating temperature.

(i) Non/plar columns (Eg.SE.30 CV-101, OV-1,SF 90), a special silylating mixture available under the name of SILYL 8 from Pierce chemical Corporation USA could be used. Two injections of 3-5 microlitre with the column held at high temperatures are sufficient. It may be advisable to detatch the column from the detector to prevent SILICA formation on it.

(ii)For certain polar-columns such as carbowax 20M (polyethylene glycol 20000) and DEGS, a few microlitre of distilled water injected into the column when it is held at 200-220^{o}C is effective in cleaning up the low-volatile materials.

ANNEX 13

A Method For Refining Dark Coloured Oil Of Ocimum gratissimum To Reduce Colour.

Oils containing large amounts of Eugenol (Phenoâs) such as oil of O. gratissimum aquire a dark brown to a blakish colour on contac⁺ with metallic iron or steel. If such contact is not prevented the quality of the oil will suffer. The only solution to this problem is to reduce the dark colouration by a chemical treatment.

A simple method used for this purpose is to agitate the oil with a small quantity of solid tartaric acid under moist conditions. A ratio of 1000 g of oilto 50 g of acid was found satisfactory when the mixture was allowed to stand for 6 hours. The oil can then be decanted through a filter to eliminate moisture and suspended matter. The filtrate will be found to be pale brown in colour, citric acid was also found to be effective but work more slowly. For large quqntities of oil costqnt stirring with solid acid in large glazed porcilain vats would be satisfactory. The process in such cases will last for several hours

ANNEX 14

.1 Standard Specifications for Essential Oils

British Standards BS 2999/1 to 15:1965

British Standards BS 2999/16 to 31 : 1972

British Standards BS 2999/32 to 43 : 1972

British Standards BS 2999/53 to 57 : 1975

International Standards Organisation ISO Specifications - Diverse numbers

Eg. Oil of lime by distillation ISO 3519-1976.

2 Methods of Analysis

Methods of Testing Essential Oils

British Standards BS 2073 : 1962

Essential Oils Sampling ISO 212-1973

Technical Data Booklet. Kalamazoo Spice Extraction Co.

P.O. Box 511 Kalamazoo Michigan USA.

3 Methods of Gas Liquid Chromatographic Analysis of Essential Oils

.1 Analytical Methods Committee :

Applications of Gas Liquid Chromatography to the Analyses of Essential Oils

Part I Analyst, (1971), 96 887

Part II Analyst, (1973) 98, 616

Part III Analyst, (1973) 98, 832

Part IV Analyst (1973) 100, 593

Part V Analyst, (1977) 102 607

Part VI Analyst, (1978) 103 375

Part VII Analyst, (1980) 105, 262

Part VIII Analyst, (1981) 106, 448

.3.2 Methods of Testing Essential Oils

British Standards BS. 2073:1976

.3.3 Gas Chromatographic and Spectroscopic Analysis of Essential Oils - Y. Massada

.4 Supplementary Reading Matter

The Essential Oils Volume I - VI by

E. Geunther (1950) New York, Van Nostrand

Infra Red Analysis of Essential Oils

Bellana to J. and Hidalgo, A. (1971) London : Heyden.

Infra-Red Spectroscopy in the Analysis of the Volatile Oils of Cinnamon. R.O.B. Wijesekera and K.H. Fonseka. J. Natn. Sci. Coun. Sri Lanka (1974) 2 (1) 35-49.

Proceedings of the Seventh International Congress of Essential Oils (1976) Kyoto, Japan.

5. List of Literature Copied to the staff of Laboratory

i. An Anthology of Essential Oils Chromatograms. A Hewlett Packard publication, Note 228-17.

ii. An Introduction to Gas Chromatography Revised by N.S.Chapman Pye-Unicam Publication.

iii. I S C O Handbook of Laboratory Data.

iv. Anlytical Chemistry Annual Review, Essential Oils and Related Products (1977).

v. Quality Control of Essential Oils in Developing Countries. by A.L; Jayewardene.

vi C R C, Review on Cinnamon: R.O.B.Wijesekera.

ANNEX 15

ANALYTICAL INSTRUMENTS INSTALLED IN THE QUALITY CONTROL LABORATORY

1. Gas Liquid Chromatograph (GLC)
   Shimadzu GC 9 A with FID and TCP coupled to a Shimadzyu C-k 6A Chromatopac computing integrator
2. High Performence Liquid Chromatograph (HPLC)
   Shimadzu LCC 6A. ISO-CRATIC Szstem with a Shimadzu SPD-6A V,UV,VIS Spectrophotometric detector combined to a C-R 6A Chromatopac computing integrator.
3. Infra- Red Spectrophometer (IR) Phillips PU 9706 with grating optics.
4. Ultra violet/Visible/Near Infrared (UV/VIS/NIR) Spectrophometer Phillips PU 8620 series single beam instrument
5. Polarimeter Polax-D (AtagoCo Japan) Digital semiautomatic analyser
6. Refractometer ABBE Type 1 T (Atago Co. Ltd. Japan) with Temeprature monitor and built Sodium Lamp.
7. TLC Densitometer Scanner (Advantec Toyo Kaisha Ltd. Japan)
   Visible Wave lenght 440-620 mm detector wilk built in Recorder and digital Printer

OTHER EQUIPMENT AND APPARATUR

1. Hydrogen Generator (Shimadzu)
2. Portable Air Compressor
3. Hand-held UV-Lamp (254+356 mm)
4. Thin Layer Chromatography Kit (Advantec Toyo Kaisha, Japan)
5. ISO-Standardized Essential Oil Collection Apparatus
6. Spares and Accesories for all instruments

Note: All instruments have stabilized power Supplies through individual power stabiliyers. They are all installed in an airconditioned room.

CATALOGUES

Library of standard reference compounds

Bottle number	Compund	Storaged where
1	a-Amyl cinnamic aldehyde	refrigerator
2	Anethol	"
3	Benzyl Acetate (extra)	Laboratory
4	Benzyl Alkohol (extra)	"
5	Citral Pure	Refrigerator
6	Citronellal	"
7	Citronellol	"
8	Dodecanol	"
9	Eucalyptol (1.8 Cineol)	Laboratory
10	Eugenol	Refrigerator
11	Geraniol (Ex Pamarosa)	"
12	Geraniol (pure)	"
13	Geranyl Acetate (pure)	"
14	Linalool	"
15	Methyl Anthranilate	Laboratory
16	Methyl Chavichol	Refrigerator
17	Paracresol (pure)	"
18	Nerolidol	"
19	Paracresol (pure)	Laboratory
20	Phenyl Ethyl Alcohol (extra)	"
21	Thymol	"

Library of standard essential oil

No.	Essential Oil
1	Cajeput Oil (Melalauca Ceucedend)
2	Celery seed oil (Apicem Graveolans)
3	Citronella Oil (CYmbopogon Nardus)
4	Citronella Java Oil (Cymbopogon Winterianus)
5	Kewda (Paudanas)
6	Lemon Grass (Cymbopogon Flexuasus)
7	Mentha Arvensis (mentha Arvensis)
8	Basilic Oil (Ocimum Basilicum)

all to be stores in refrigerators.

ANNEX 17

RECOMENDED ACCESSORIES AND REQUIREMENTS

Requirments for Gas Liquid Chromatography

1. Coated GC Column Packing Materials :

Carbowax 20M,	10% w/w on Chromosorb W	100 g
Silicone SE 30,	5% w/w on Chromosorb W	100 g
FFAP	10% w/w on Gas Chrom Q	50 g
OV 101	5% w/w on Gas Chrom Q	100 g
OV 275	10% w/w on Gas Chrom Q	50 g
SP 2340	5% w/w on Gas Chrom Q	50 g

All in 60/80 mesh or 80/100 mesh sizes .

2. Liquid Stationary Phases :

Carbowax 20M	100 g
SE 30	100 g
OV 101	25 g
FFAP	25 g
OV 225	10 g
DEGS (Diethyleneglycol succinate)	50 g

3. Inert Packing solid support :

Chromosorb W (AW,DCMS)	60/80 mesh	200 g
Chromosorb W (AW,DCMS)	80/100 mesh	200 g
Gas Chrom P	60/80 mesh	200 g
Gas Chrpm Q	80/100 mesh	200 g

Silylating Agents :

Trimethyl silyl Chloride	50 ml
Hexamethyl Disilazine (HMDS)	50 ml
Silyl 8 (PIerce Chemical Co) (Column conditioner)	25 ml

Microlitre syringes :

SGE	0,5	microliter (P/N 020701)	2nos
SGE	1,0	" (P/N 020705)	2nos
SGE	5,0	" (P/N)	2nos
SGE	100	"	1nos

Materials for Thin Layer Chromatography:

Silicagel G (TLC)	1 kg x 2 nos
Alumina G (TLC)	1 kg x 2 nos
Vanillin (Spray Reagent)	100 g
Anisaldehyde (Spray Reagent)	100 ml
Petroleum Ether AR 60/80	2 1/2 L
Toluene AR	2 1/2 L
DI-Isopropyl ether GPR	2 1/2 L
Acetone AR	2 1/2 L

HPLC

Accessories and other Requirments :

1. GUARD COLUMNS :

2 each of packing, C8, C18 _Octadecyl Silane 'ODS) and Phenyl type

2. SOLVENT FILTRATION APPARATUS :

1 litre Buchner flask with ground glass neck joint.
1 Split 2 pieces buchner funnel to fit flask.
1 Box of 0.5 micrometer membrane filter disc.
1 Spring clip to fix funnel valves together.
1 Rubber vacuum hose.

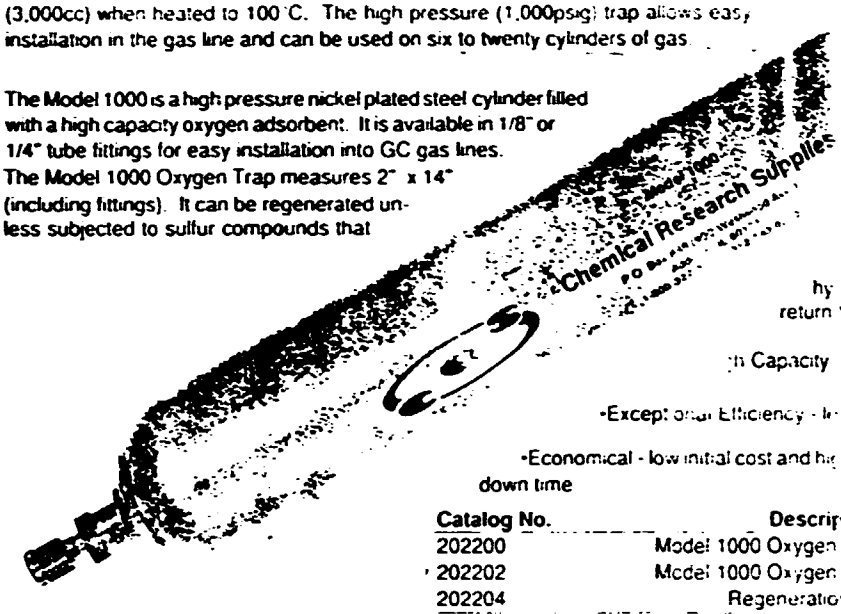
3. SOLVENTS HPLC GRADE :

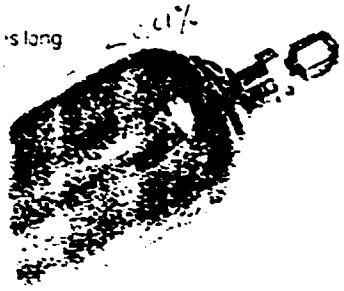
Hexane ,Cyclohexane ,Dichloromethane	2L each
Ethyl acetate , Chloroform	2L each
Methanol , Isopropanol	5L each
Acetonitrile , Tetrahydrofuran	5L (the later1L)

OXYGEN TRAPS

NEW!!! - MODEL 1000 OXYGEN TRAP

Trace oxygen can ruin your column before you know it. The Model 1000 Oxygen Trap provides long term oxygen removal for chromatographers using capillary columns. The high capacity allows months of use without worry. If desired, the trap's capacity can be increased 3 times (3,000cc) when heated to 100°C. The high pressure (1,000psig) trap allows easy installation in the gas line and can be used on six to twenty cylinders of gas.





The Model 1000 is a high pressure nickel plated steel cylinder filled with a high capacity oxygen adsorbent. It is available in 1/8" or 1/4" tube fittings for easy installation into GC gas lines. The Model 1000 Oxygen Trap measures 2" x 14" (including fittings). It can be regenerated unless subjected to sulfur compounds that poison the adsorbent. Regenerati requires high temperature treatment w hydrogen. Instructions are included or you c return the trap to CRS for regeneration.

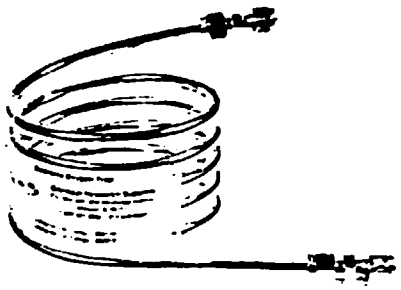
•High Capacity - over 1 liter of oxygen can be removed

•Excep: onal Efficiency - less than 50ppb oxygen content at the outl

•Economical - low initial cost and high capacity gives months of use and reduc down time

Catalog No.	Description	Pric
202200	Model 1000 Oxygen Trap, 1/8" fittings	$98.0
202202	Model 1000 Oxygen Trap, 1/4" fittings	98.0
202204	Regeneration Service	25.0

ECONOMY OXYGEN TRAP

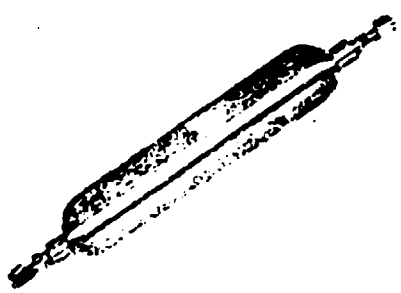


- •High capacity
- •Ready for use
- •Made of nickel plated copper

The Economy Oxygen Trap is selective to oxygen and sulfur compounds. It will reduce th oxygen level in the carrier gas to less than 0.1ppm. The capacity is enough to scrub th oxygen out of three 200 cubic foot cylinders. The Economy Oxygen Trap is suitable for us with capillary columns to protect the thin liquid phase. The trap is supplied with 1/4" or 1/ fittings and can be regenerated repeatedly. Regeneration service is available throug Chemical Research Supplies.

Catalog No.	Description	Price
202210	Economy Oxygen Trap, 1/8" fittings	$49.00
202212	Economy Oxygen Trap, 1/4" fittings	49.00
212214	Regeneration Service	18.00

HIGH PRESSURE OXYGEN TRAP



- •Made of stainless steel (Type 304)
- •1800psig rating
- •Can be regenerated
- •Ideal for high flow applications

The High Pressure Oxy ap can efficiently remove 650cc of oxygen. This is equal t 12 cylinders of inert ga larger diameter makes it capable of much larger flows tha other oxygen traps. In re use and regeneration instructions.

Catalog No.	Description	Price
202250	Pressure Oxygen Trap, 1/8" fittings	$185.0
202252	Pressure O p, 1/4" fittings	185.0
202254	Regen vice	27.0

Address: CHEMICAL RESEARCH
P.O. Box 868 Greenwood Ave
ADDISON IL 60101

OXYGEN TRAPS

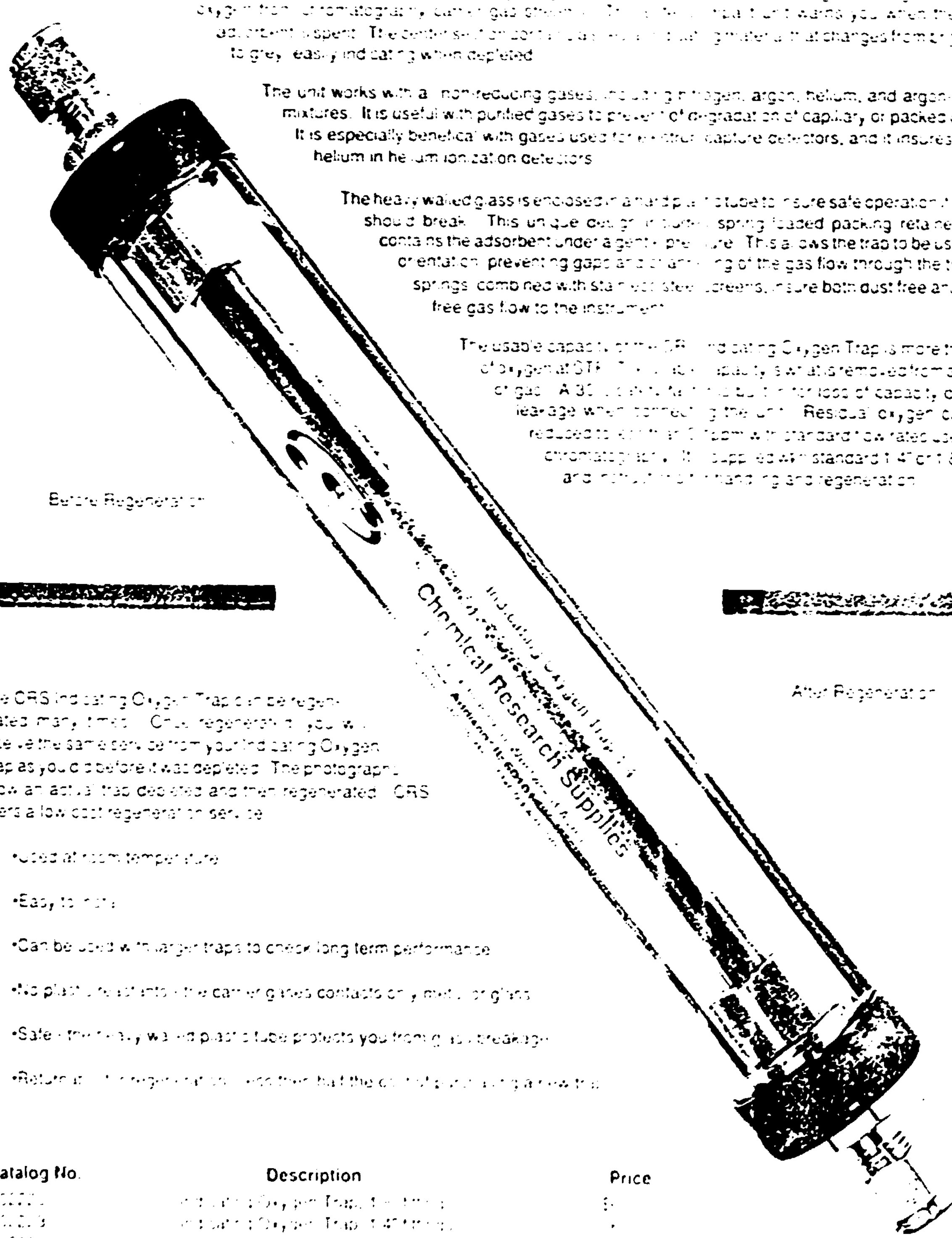
INDICATING OXYGEN TRAP

The Chemical Research Supplies Indicating Oxygen Trap [illegible] designed to remove oxygen from chromatography carrier gas streams. [illegible] unit warns you when the oxygen adsorbent is spent. The [illegible] changes from bright green to grey, easily indicating when depleted.

The unit works with all non-reducing gases, including nitrogen, argon, helium, and argon-methane mixtures. It is useful with purified gases to prevent degradation of capillary or packed columns. It is especially beneficial with gases used for electron capture detectors, and it insures purity of helium in helium ionization detectors.

The heavy walled glass is enclosed in a hard plastic tube to insure safe operation if the glass should break. This unique design [illegible] spring loaded packing retainers which contains the adsorbent under a gentle pressure. This allows the trap to be used in any orientation, preventing gaps and channeling of the gas flow through the trap. The springs, combined with stainless steel screens, insure both dust free and oxygen free gas flow to the instrument.

The usable capacity of the CRS Indicating Oxygen Trap is more than [illegible] of oxygen at STP. [illegible] capacity is what is removed from a [illegible] of gas. A 30 [illegible] for loss of capacity due to air leakage when connecting the unit. Residual oxygen content is reduced to [illegible] ppm with standard flow rates used in gas chromatography. It is supplied with standard 1/4" [illegible] fittings and [illegible] handling and regeneration.



Before Regeneration

After Regeneration

The CRS Indicating Oxygen Trap can be regenerated many times. Once regenerated, you will receive the same service from your Indicating Oxygen Trap as you did before it was depleted. The photographs show an actual trap depleted and then regenerated. CRS offers a low cost regeneration service.

- Used at room temperature
- Easy to [illegible]
- Can be used with larger traps to check long term performance
- No plastic [illegible] the carrier gases contacts only metal or glass
- Safe [illegible] heavy walled plastic tube protects you from glass breakage
- Return [illegible] regeneration [illegible] half the cost of purchasing a new trap

Catalog No.	Description	Price
[illegible]	Indicating Oxygen Trap [illegible]	$[illegible]
[illegible]	Indicating Oxygen Trap 1/4" [illegible]	[illegible]
[illegible]	Regeneration Service	[illegible]