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**PRODUCTION OF PHARMACEUTICAL MATERIALS FROM
MEDICINAL AND AROMATIC PLANTS**

DP/TUR/88/001

TURKEY

**Technical Report :
Findings and Recommendations***

**Prepared for the Government of Turkey
by the United Nations Industrial Development Organization
acting as executive agency for the United Nations Development Programme**

**Based on the work of Mr. Jan Karlsen
Pharmacist-analytical chemist**

**Backstopping Officer: ROB WIJESEKERA
Chemical Industries Branch**

United Nations Industrial Development Organization

Vienna

* This document has not been edited.

1. Introduction

My visit to TBAM this time (sep/oct 88) was the first of two visits intended as a follow-up of my previous assignments under DP/TUR/83/003. The main objective was to advise in good laboratory practice as well as carrying out the intentions of my job description. When I left about one year ago on my previous assignment a list of recommendations was left behind to be followed by the employees of the quality control section. In addition to my job description I was also expected to participate in the planning and execution of the international training course (TRUMAP) due to take place in Eskirşehir during my stay. Here I was to give lectures and lead the practical instrumental demonstrations.

During the first phase of the TBAM project I was concerned with the design of the laboratory, purchase and installation of instruments and fundamental training of the personnel in the handling of the equipment. The first phase can be regarded as an establishing period for the laboratory, the second phase may be regarded more of the organization of analytical data and projects. To determine how the recommendations I left 1987 have been followed, the various principal recommendations in my previous report have been examined; the findings are discussed in the following paragraphs. My comments on the quality of essential oils produced in Silifke is introduced in part 5.

2. Activities and outputs (Ref., Appendix 2)

- 2.1. By accepting phase 2 of the project support to TBAM is given. This support will in any case assure the reaching of international standard of the analytical laboratory at the end of the phase 2.
- 2.2. The library is still very inadequate and still will seriously limit the future activities of TBAM. Most strongly felt is the need for relevant journals. The more common journals used for advertisements and reporting by the perfumery industry is most certainly needed if TBAM is to give advice on the production of raw materials for perfume and aroma formulation.

A subscription as well as purchase of the issues of the previous five years is recommended.

- 2.3. Industrial standards of essential oils and "pure" substances have been obtained. This has given TBAM the necessary "library" of substances.
- 2.4. The training of staff members in the use of English is an on-going process. This is helped by the visits of the technical experts. The training program (TRUMAP) also to a great extent forced the staff to use the English language.
- 2.5. An HPLC system complete with automatic injection system, fluorescence detection system and gradient pumping system has been ordered. This equipment still has my highest priority for TBAM. Another gas chromatograph or a GC/MS system would be second priority. The choice of which much depends upon the future activity of TBAM. For research purposes a GC/MS may be necessary. Research component must increase with the GC/MS purchase.
- 2.6. The terpene standards are stored in the refrigerator and controlled regularly.
- 2.7. The TBAM staff has been given an example of a standard report on an analytical method developed and tested by the quality control laboratory (Appendix 13). Larger reports exist in the form of M.Sc. theses. Standard formats on technical reports has been discussed but no definite action taken.
- 2.8. During 1987/1988 26 fresh distilled essential oils were analysed. A better GC-capillary column prompted the rerun of some of the samples. Essential oil containing seeds as well as 15 new oils will be investigated 1988/1989. By September 1989 the manuscript for a data collection on essential oils from Turkey of potential commercial value can be expected.

- 2.9. Standard pages for the data collection mentioned in 2.8. are given as model (Appendix 5).
Manuscript expected to be ready by September 1989.
- 2.10. A brochure has been made, but another one is strongly advised as it is meant to attract industrialists and scientists. Professional lay-out is required as well as glossy paper background. This is a most important part of the marketing of the services of TBAM. Most laboratories in Western Europe offering analytical services have similar brochures and this is regarded as a necessity towards potential customers.
- 2.11. Project meetings have not yet materialized in TBAM although encouraged by the experts.
- 2.12. A project management course is strongly recommended for the director of TBAM. More organizational skill is needed.

3. Analytical laboratory.

The quality control laboratory and the phytochemical laboratory are now functioning well with the existing equipment. The throughput of analyses of the pilot plant samples is satisfactory but the GC is operating at maximum efficiency. The time available for development and research work is limited. A doubling of the instruments for GC and HPLC-analysis will be a natural extension of the analytical facilities. During the last year a large number of analyses were carried out in the quality control laboratory. Some of the equipment is idle but that is normal as only a limited number of projects can be handled at the same time.

The analysis of terpenes and essential oils has improved during the last year as new columns and new electronic integrators were put to practical use. The separation achieved is now fully compatible with that achieved in perfumery factories. Correspondingly reliable certificates can therefore be issued when required.

The most important issue at the moment for the quality control laboratory is the rational organization of the collection of analytical data. More time must also be given to long-term planning to allow the staff time for method development and research. Unless care is taken 100% of the time is spent at routine analyses.

The staff is taking well care of the laboratories and the analytical instruments. My recommendation of a "dustfree" environment has been followed.

Computers and software have been purchased and the GC and HPLC instruments are both equipped with two-channel integrators. This allows for easy extension of instruments.

Only designed personnel are allowed to run the instruments and log-books follow each separate piece of equipment.

Considering the workloads on the quality control laboratory I would consider one extra staff member for the analytical laboratory to be necessary. Apart from the one extra person I find the number of personnel adequate for this size of an analytical laboratory.

I also recommend better use of the many personal computers available for data storage of analytical results.

4. Workplan 1988/1989

On the basis on the workplan for 1987/1988 the Director of TBAM and the UNIDO experts (N.Bisset, M.B.Narasimha, J.Karlsen) have discussed a new workplan for 1988/1989. We have taken into account possibly new projects originating in recent industrial contacts, routine analysis of samples from ongoing projects, research projects and method development. This time it was difficult to foresee dates for expected reports so only a list of expected outputs was produced. This is found in Appendix 10. The workplan as agreed upon will give the staff of the analytical laboratory ample opportunities to gain further experience in quality control analysis. The number of analysis to be carried out will also require some administration as to the rational storage of analytical data.

The workplan contains a number of activities that calls for long-term planning and regular meeting between staff assigned to the pilot plant and to the quality control laboratory.

5. Quality Control of Essential Oils produced in the Silifke factory

During the SIS project (revival of the essential oil producing factory in Silifke) the output from the factory will be a certain volume of essential oils. For the in-process control a quality control laboratory will be necessary.

For the quality control of essential oils at the production site, a control of the physico-chemical properties of the oil produced is sufficient. This is limited to the determination of the amount of oil contained in the plant material (bench scale), the determination of optical rotation and the determination of refractive index. In some cases acid equivalents and ester equivalents are measured. For this quality control a small laboratory is sufficient. More sophisticated analyses like gas chromatography, UV and IR spectroscopic measurements is recommended to be carried out elsewhere. In this specific case TBAM in Eskicehir has the necessary facilities for these analyses. There is a daily bus service Eskicehir - Mersin - ~~Eskicehir~~ passing Silifke so samples can reach TBAM easily one day after distillation if necessary.

List of equipment necessary for quality control, Silifke.

- 1) Titration unit (burette, etc...) x 2
- 2) Clevenger distillation unit with heating mantle x 2
- 3) Optical rotation instrument (Optical Activity Ltd)
- 4) Abbé refractometer (Shimadzu)

The technical specifications as well as prices for this equipment can be taken from TBAM.

6. Additional equipment recommended.

This list of recommended equipment is based on the workplan, on projects in cooperation with Turkish industry and on what the expert (J.K.) regards as a natural extension of the laboratory facilities.

- 1) 1 GC (capillary system)
- 2) 1 HPLC (gradient system, refractive index, fluorescence, UV-VIS detectors)
- 3) 1 Automatic injection system for HPLC
- 4) 1 Titration system (TR 151, Schott Geräte)
- 5) 1 Dissolution tester for solid dosage form pharmaceuticals (Sotax, Switzerland)
- 6) 1 Disintegration tester for solid dosage form pharmaceuticals (Erweka, Germany)
- 7) 1 GC-MS system (Shimadzu, Japan)
- 8) 5 Low dead-volume solvent resistant chromatographic columns (Pharmacia, Sweden)

These additional equipment has not been listed according to priority. This will depend upon long-term planning of the activities of TBAM. As mentioned in a previous paragraph the number of analyses carried out in the quality control laboratory has reached its maximum - therefore a doubling of the GC and the HPLC equipment seems logical (1), 3), 2)). If TBAM is moving into investigation of pharmaceutical dosage forms based on plant raw material, the most essential test instruments are necessary (5), 6)).

For the more simple analyses like titration of acids and bases, a titration system will be an asset to the laboratory. Standardized analyses will then be performed. (4)

For preparative purposes in the phytochemical laboratory low dead-volume chromatographic columns are non-existing. Efficient isolation of interesting compounds from plants is usually done by column chromatography and therefore modern columns are needed(

A laboratory specializing to some extent in essential oil research do need a GC-MS instrument. Since such a system requires more background and a well-planned organization of projects to be utilized to a full extent, the purchase of a GC-MS system must be coupled to the long-term planning of the activities of TBAM. If a GC-MS system is purchased it is certainly recommended that an expert in analytical chemistry is fielded in 1989 and 1990 (Short term) (7).

ii. Recommendations.

1. The Medicinal Plant Research Center (TBAM) of the University of Anatolia has started out well but will need further support to get all the way to international standard.
2. The library is inadequate and needs further strengthening both in books and journals. Several standard textbooks of chromatography is lacking.
3. The laboratory badly needs industrial standards of essential oils. These can also be bought from chemical companies. It is realized that this may take some time to procure. In the meantime some of the most important terpenes should be run as standards.
More training for the local staff in the use of English
4. is necessary.
5. Top priority for new equipment should be another HPLC with fluorescence detector, and a fluorimeter.
6. The standards, kept at low temperature in the refrigerator, must be controlled regularly.
7. Regular technical reports should be made by means of the IBM PC AT to familiarize the staff with the use of word processing and English.
8. It is highly recommended that 40 - 50 essential oils of Turkey be analyzed by standard methods for their content of main constituents.
9. The above-mentioned results should be published as a product of TBAM for the benefit of potential customers of Turkish essential oils.
10. The making of a brochure to market and inform about the activities of the center.

11. Encourage the staff to have regular meetings and discuss their projects.

12. The director needs to have some training in project management. The change from academic life to a research laboratory requires more effective administrative skills. I would therefore strongly recommend a monthly course in project management.

TBAM ANALYTICAL PROCEDURE

No : 001

HPLC determination of Capsaicin In Capsicum Oleoresin

Summary:

HPLC conditions for the quantitative estimation of capsaicin have been evaluated as part of a project concerning the capsaicin content of dried Turkish Capsicum.

**Eskişehir
October 1988**

1. Instrumental conditions:

HPLC : Shimadzu LC-5A

Mobile phase : 1. Dioxan : H₂O (46:54), or
2. MeOH : H₂O : AcOH (65:34:1)

Temperature : Ambient (20⁰C)

Column : C₈, reversed phase

Detector : 280 nm

Flow rate : 1 ml/min

2. Sample Preparation:

Sample size : 10 g

Extraction procedure :

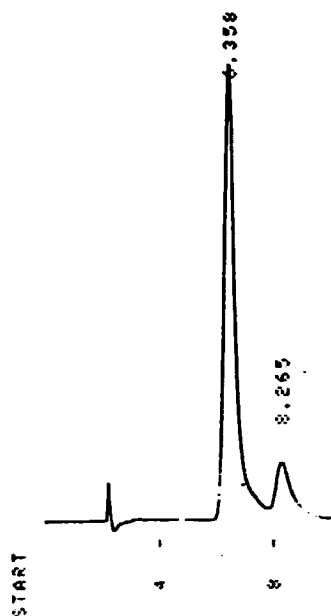
- a) Maceration: 200ml %96 Ethanol, 7 hours.
- b) Soxhlet: %96 Ethanol, 7 hours.

Solvent removed under reduced pressure.

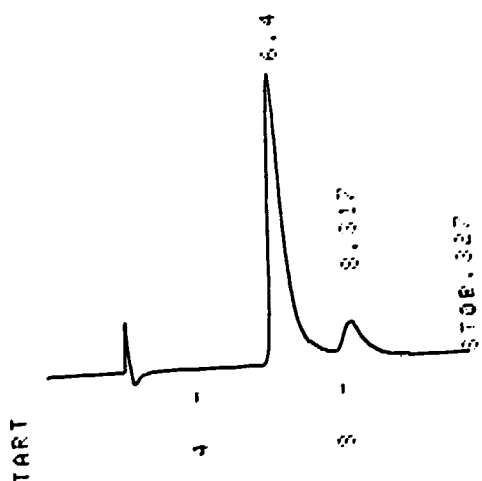
Dilution : 5-10 mg residue dissolved in 1ml
methanol and filtered.

HPLC : 20 ul of sample solution

3. Model chromatograms:



Chromatogram of standard capsaicin*



Chromatogram of crude capsicum oleoresin*

*Mobile phase: MeOH:H₂O:AcOH (65:34:1)

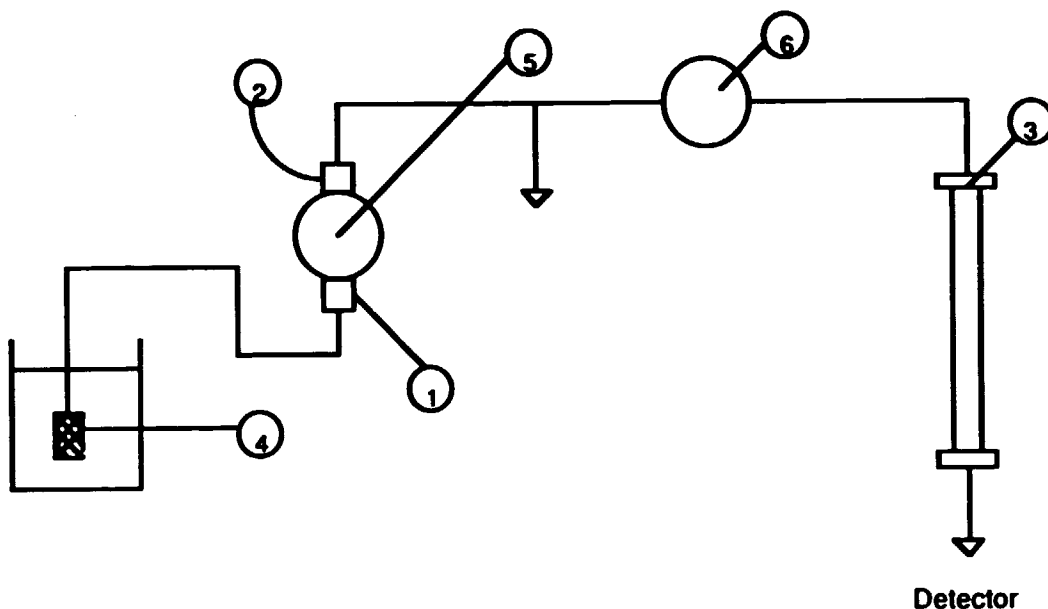
Appendix ...

Specific outputs 1988/1989

- 1. GC-analysis of 15 essential oil isolated from plants collected in Turkey**
- 2. GC-analysis of oils isolated from commercially available seed material**
- 3. Quality control analyses of essential oils originating in the Silifke production unit**
- 4. Developing a HPLC-method for the Gypsophila saponins**
- 5. Developing a HPLC-method for the naphthoquinones in Arnebia species.**
- 6. Analysis of the carotenoids in Capsicum species**
- 7. Routine analysis of aescin**
- 8. Quality Control analysis of Rose oil**
- 9. Analysis of fusel oil fractions**
- 10. Routine analysis of /Pine oil fractions**
- 11. Analysis of alkaloids from Datura stramonium**
- 12. Preparing a booklet on 40-50 essential oils of Turkey of potential commercial volume**
- 13. Analysis of purified Styrax rosin**
- 14. Routine analysis of Origanum oil**
- 15. Routine analysis of Glycyrrhiza glabra extract**

Checklist for HPLC

PUMP CANNOT DELIVER MOBILE PHASE AT A CERTAIN BACKPRESSURE



These items must be cleaned by ultrasonic treatment in water and methanol according to the following priority:

- ① Inlet valve
- ② Outlet valve
- ③ End filter in column
- ④ Solvent filter
- ⑤ Quartz piston in pump head (follow instructions in manual)

VERY CAREFUL

- ⑥ Injector

Appendix 12

Journals to be recommended to be bought by the TBAM:

- 1. Fette, Seifen and Anstrichmittel**
- 2. Perfumery and Cosmetics**
- 3. Dragoco Report**
- 4. The Chemical Manufacturer**
- 5. Soap, Perfumery and Cosmetics**
- 6. Flavour and Fragrance Journal**
- 7. Parfums, Cosmetiques et Savons**
- 8. Perfumer and Flavorist**
- 9. Natural Products Report**

Pinus halepensis Miller (Pinaceae)

Fl. Turkey 1: 75 (1965)

Collection:

Number: Başer and Karlsen 1

Locality: İçel

Date: 18-9-88

Occurrence:

Locally abundant

Processing:

Plant part: Needles

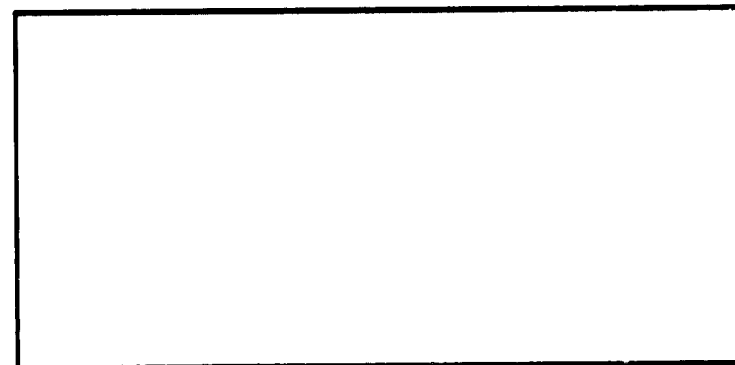
Distillation: Hydrosteam

Solvent extraction: -

Headspace analysis: -

Date: 19-9-88

Yield: 1.1%



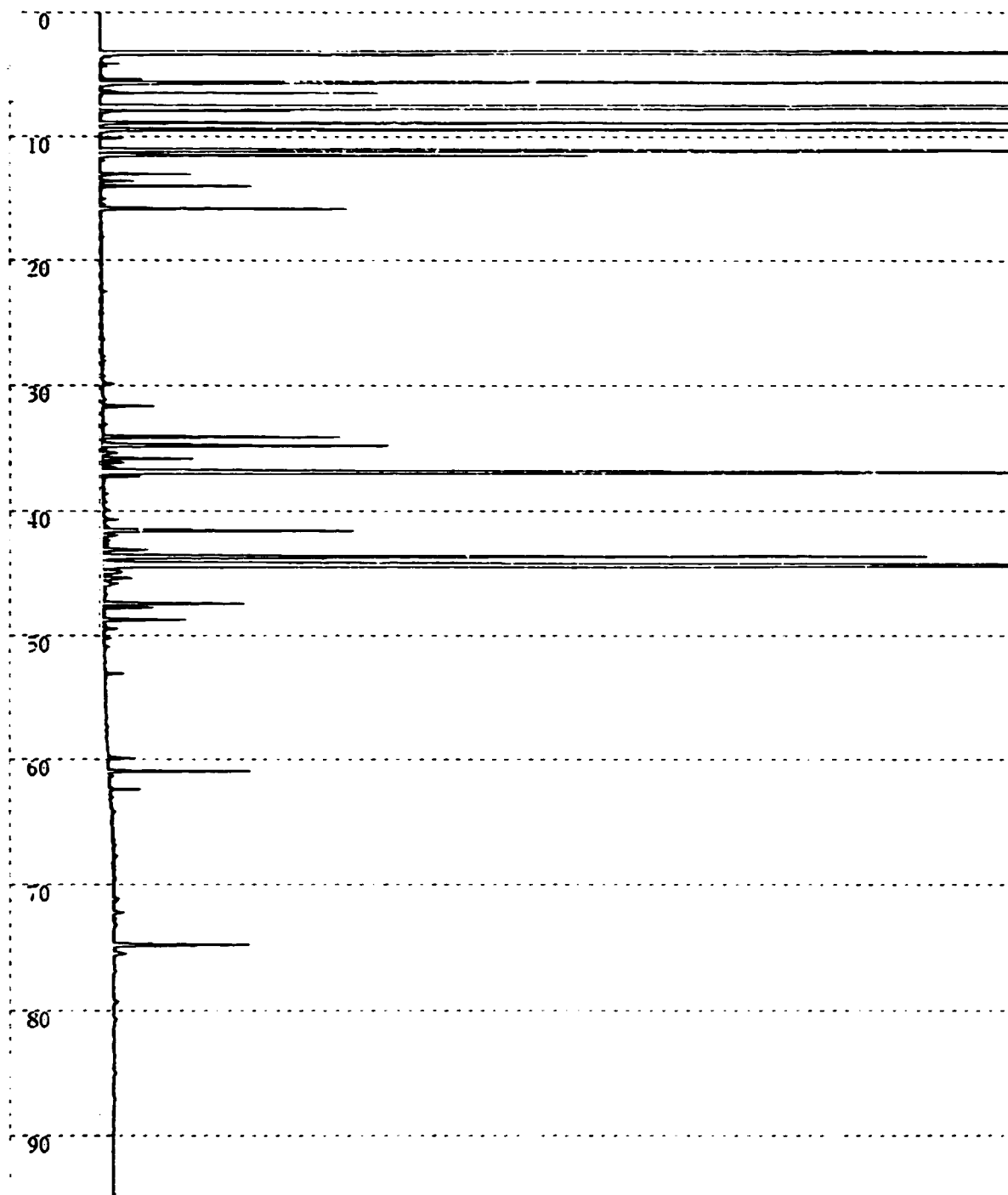
Main constituents: α -Pinene

β -Pinene

Bornyl acetate

Comments: Typical Pinus oil

References:



Oil from Pinus Halepense
Fused silica capillary 50m
Thermon 600T
Shimadzu
70°-10 min
2°/min.
180°-30 min.