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

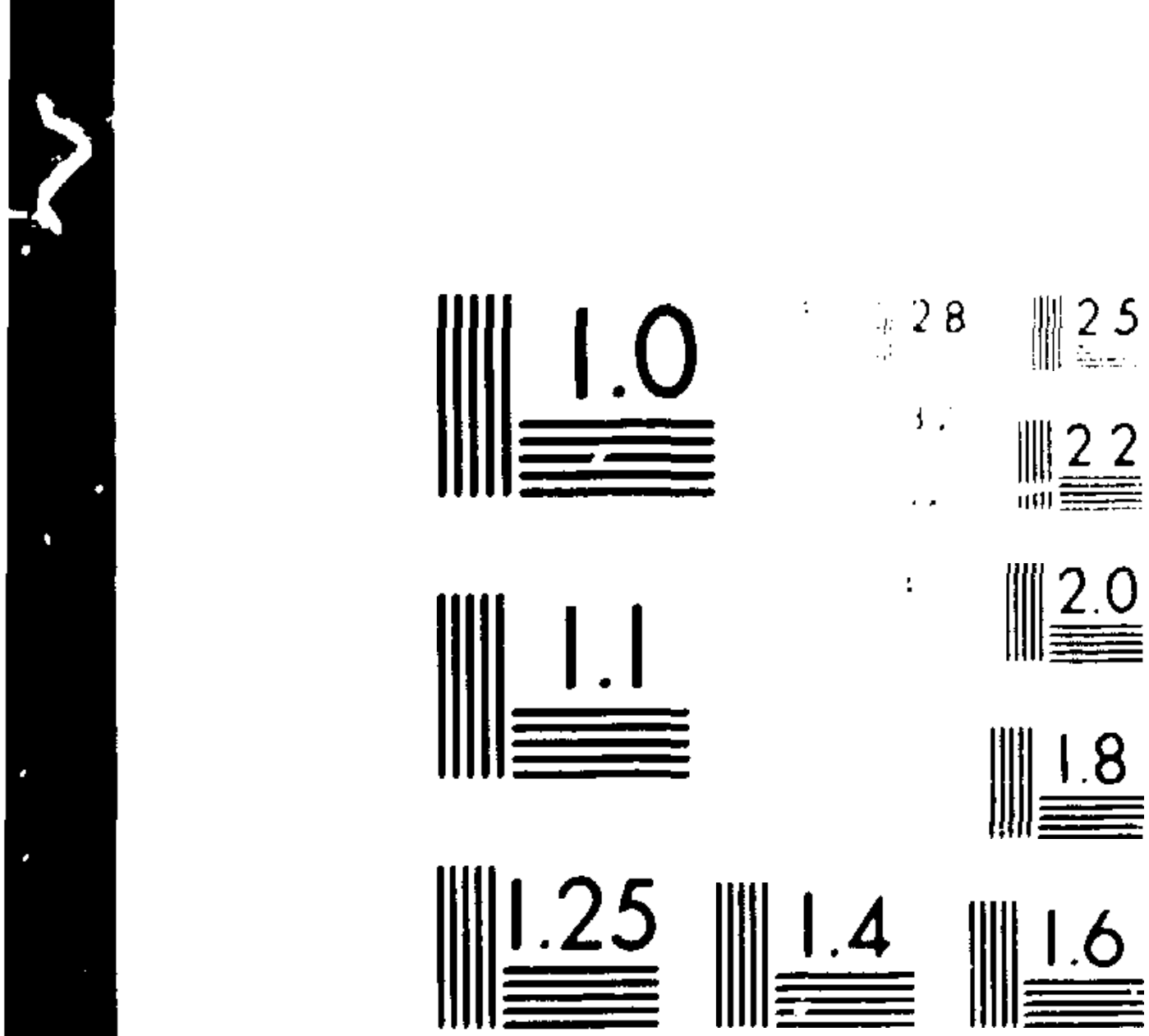
FAIR USE POLICY

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


1.0
2.8
2.5
2.2
1.1
2.0
1.8
1.25
1.4
1.6

RESTRICTED

11603

DP/ID/SER.A/352
25 March 1982
English

Cuba. THE QUALITY CONTROL OF HECOGENIN AND ITS PRODUCTS.

SI/CUB/75/803

CUBA

Technical report *

Prepared for the Government of Cuba
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Gerald Blunden,
expert in the quality control of hecogenin and its products

United Nations Industrial Development Organization
Vienna

003080

* This document has been reproduced without formal editing.

V.82 23980

ABSTRACT

This is the report of a mission to Cuba entitled "The Quality Control of Hecogenin and its Products", undertaken between June 28th and July 19th, 1981 (SI/CUB/75/803/11-02/32.1.D) in conjunction with the project "Establishment of a Pilot Plant for the Production of Hecogenin" (SI/CUB/75/803/11.01). The United Nations Industrial Development Organization (UNIDO) was the executing agency.

Samples of Agave fourcroydes juice and "coffee grounds" produced at the pilot plant at Cardenas were analysed for their contents of hecogenin and tigogenin by a gas-liquid chromatographic method. Another analytical method, using high performance liquid chromatography, was discussed, as well as related methods, using the same apparatus, for the analysis and quality control of drugs derived from hecogenin. As all the products that are to be produced can be analysed by high performance liquid chromatography, it is recommended that this equipment, along with modern gas-liquid chromatographic apparatus, be supplied for the work in Cuba. As it is necessary that the equipment should be maintained in working order, it is recommended that either a Cuban technician be sent for suitable training or an expert be sent to Cuba to teach technicians how to instal, maintain and repair the apparatus supplied.

Analytical results from the juice and "coffee grounds" produced at the pilot plant at Cardenas in July showed unacceptably high levels of tigogenin. The possibility of seasonal variations in the relative proportions of tigogenin and hecogenin has to be investigated as it may be uneconomic to process juice for "coffee grounds" and hecogenin at certain times of the year.

Studies in the chemical transformation of hecogenin into corticosteroids are progressing. Other Cuban materials that have potential as starting materials for conversion into steroid drugs are being investigated also. Considerable quantities of sterol from sugar cane waste are available and this is being studied as a precursor for the production of contraceptive steroids.

CONTENTS

INTRODUCTION

A. Project background

In August 1976 Dr. Gerald Blunden visited Cuba to evaluate the potential economic use, as sources of steroids, of several plants that had been investigated in the country (SI/CUB/75/001/11-03/4). Of the plants available at the time it was found that Agave fourcroydes contained hecogenin, an ideal precursor for the production of corticosteroids. This sapogenin was found to be present in sufficient quantity to make the plant a possible industrial source of hecogenin and, as A.fourcroydes is cultivated in Cuba to supply the fibre henequen, sufficient leaves are available to yield economic quantities of hecogenin. The major recommendations in the report were that a pilot plant be established for the production of crude hecogenin concentrates ("coffee grounds") and that a quality control laboratory should be started for the analysis of hecogenin.

As a result of the recommendations made, the Cuban Government decided to establish a pilot plant for the production of crude hecogenin concentrates, which could then be used for the extraction of hecogenin. The second phase of the project (SI/CUB/75/008/A/11/37/30408), executed by UNIDO/UNDP, was titled "Instalación de una planta piloto para la producción de hecogenina para la industria farmacéutica en Cuba". Ir. J.G. Kroonen was proposed for the project and his candidature was accepted by the Cuban Government in April 1979. He made a preliminary visit to the country from May 15th to June 3rd, 1979 and his report "Establishment of Pilot-Plant for the Production of Hecogenin in Cuba - Report on fact-finding mission to Cuba" (SI/CUB/75/803/11-01/32.1.D) was summarised as follows:

(1) The juice expressed from the butt-end waste of the decortication process of the leaves of A.fourcroydes is a satisfactory raw material for the production of a crude hecogenin concentrate with a low content of the unwanted tigogenin.

(2) The potential juice production from the henequen estates was estimated at 15,000,000 litres per annum, with an average content of 0.7 g hecogenin per litre.

(3) Inconclusive results were obtained from small scale experiments carried out using the foaming process for the recovery of hecogenin from the juice. Therefore it was proposed to start hecogenin production with a 2-step acid hydrolysis of undiluted juice, allowing for the possibility to change to the foaming process in the future.

(4) A flow sheet and a provisional lay-out of the pilot plant, accommodating the 2-step acid hydrolysis process and a small foaming section were prepared.

(5) The factory site was planned near the henequen production centre at Cardenas.

(6) Lists of important equipment which were to be imported and which were to be constructed locally were drafted.

(7) A provisional working programme for the EPROYIN engineers who will supervise the building activities was made.

(8) Suggestions were made to find outlets for the crude hecogenin concentrates known as "coffee grounds" during the period that Cuba will require to build its own steroid industry.

(9) A programme of activities to be carried out by the Laboratorio Dr. Mario Muñoz was prepared, the principal one of which was to produce hecogenin from the "coffee grounds".

In 1980 and 1981 Ir. Kroonen returned to Cuba for the establishment of the pilot plant at Cárdenas for the production of crude hecogenin concentrates. As part of the same overall project, a second post was created for an expert in the quality control of hecogenin and its products (SI/CUB/75/803/11-02/32.1.D). This latter programme, carried out by UNIDO/UNDP was commenced on June 28th and finished on July 19th, 1981.

B. Objectives of the project

1. Overall objective

To establish a pilot plant for the production of "coffee grounds" for the extraction of hecogenin.

2. Immediate objectives

(1) To prepare the list of equipment required for the quality control of hecogenin and its products.

(2) To establish the quality control unit.

(3) Work out the methods of analysis.

(4) Train Cuban technicians.

(5) Introduce methods of analysis for the quality control of drugs from hecogenin, such as corticosteroids.

The objectives were achieved during the assignment, except that many of the analytical methods depend on high performance liquid chromatography. This apparatus at the Laboratorio Dr. Mario Muñoz is not functioning and is not ideally suited to all the analyses required.

I. FINDINGS

A. Quality control of hecogenin and drugs derived from hecogenin, such as corticosteroids

For the routine analysis of the contents of hecogenin and tigogenin in the leaves and juice of Agave species and the crude sapogenin concentrates known as "coffee grounds", a gas liquid chromatographic method has been devised by Cripps and Blunden (Steroids 1978, 31, 661-669). This method was supplied to the Laboratorio Dr. Mario Muñoz shortly after publication and has been in use by them. The method was modified in the Laboratorio as the gas liquid chromatographic equipment available was not capable of temperature programming. Very good results are obtained and the operators are highly competent. The main problem is that the machine being used is now obsolete, spare parts are lacking and its life span is limited.

Another method of analysis for hecogenin and tigogenin is a high performance liquid chromatographic procedure (J.W. Higgins - J.Chromatography 1976, 121 329-334). This method has the disadvantage that the sapogenins have to be converted into derivatives so that they can be detected and estimated using an ultraviolet light recorder. However, a very recent paper by Tal and Goldberg (Journal of Natural Products 1981, in press) shows that it is not necessary for derivatives to be made if a refractive index recorder is used. These methods have been tried in my laboratory and shown to be reliable.

It is the aim of the Ministry of Health (MINSAP) to convert hecogenin into corticosteroids or medicinal use. The major corticosteroid drugs can be routinely tested for purity using high performance liquid chromatographic procedures. The methods are proven and some are in routine use in my own laboratory.

It is my opinion that high performance liquid chromatography can be used for the simple, efficient and accurate estimation of all the steroids to be handled in this project from hecogenin and tigogenin to final steroid drugs, such as corticosteroids and contraceptives. Some high performance liquid chromatographic apparatus is available in the Laboratorio Dr. Mario Muñoz, but does not function at present. Moreover, the ultraviolet light recorder fitted to the machine is fixed at one wavelength, whereas, for the range of compounds that have to be estimated, a number of wavelengths are required. Also to assay hecogenin and tigogenin, without making derivatives, requires the use of a refractive index recorder. It is

my opinion that it would be of considerable benefit to the steroid programme in Cuba if a complete set of high performance liquid chromatographic apparatus be purchased and supplied to the Laboratorio Dr. Mario Muñoz. In fact, without such apparatus, I cannot see how effective quality control of corticosteroids produced can be achieved satisfactorily. Routine analysis of hecogenin and tigogenin can be continued at present using the gas liquid chromatographic procedure, but the life of the present apparatus is probably short. If funds permit, it would be of great value if replacement gas liquid chromatographic apparatus could be supplied also to the Laboratorio Dr. Mario Muñoz. The apparatus in current use is an obsolete model and spare parts would not be available when the machine breaks down.

One major problem in the supply of high performance liquid chromatographic and, to a lesser extent, gas liquid chromatographic apparatus to Cuba is that most of the best equipment is manufactured by U.S. companies and, therefore, shipment to Cuba is difficult. It is probable that equipment manufactured outside of the U.S. would have to be found.

A suggested list of equipment necessary for the routine analysis of hecogenin and steroidal compounds derived from it is given later (page 8). First priority would be the high performance liquid chromatographic apparatus. It is necessary to purchase a good supply of spare parts along with the basic equipment, as obtaining replacements from Cuba is difficult.

It is essential, in my opinion, that <u>either</u> a technician from Cuba be sent on a course of about 2 months to learn how to use, maintain and repair the equipment, <u>or</u> an expert be sent to Cuba for about 1 month to teach technicians how to instal, use, maintain and repair the machines. Without such training the apparatus could soon be non-functional (see annex 2, page 14).

B. List of equipment required for the quality control of hecogenin and its products

1. High performance liquid chromatographic apparatus

(in brackets the equipment manufactured by Waters Associates is listed and the prices (in U.S. $) are those quoted by this Company in the United Kingdom. Equivalent equipment would have to be obtained, as Waters Associates is a U.S. Corporation and will not supply Cuba. The Cuban Laboratory has some apparatus manufactured by Hewlett Packard, but I have been notified by this Company that it is unable also to sell equipment destined for Cuba)

	U.S. $
2 x Solvent Delivery Systems [110 or 220 V 60 cycles/min] (Model 6000 A) each 4885	9770
Injector (Model U6K Universal injector)	1794
Differential Refractometer (R-401 Differential Refractometer)	4650
Variable Wavelength Absorbance Detector (Model 450)	7300
Dual Pen Recorder (Philips PM 8252)	2000
4 x 30 cm. columns - octadecyl alcohol on silica (μ-Bondapak C-18 column) each 270	1080
Column packing equipment (e.g. Magnus Scientific)	1200
Total	27,794

*2. Gas-liquid chromatographic apparatus

Gas Chromatograph (PU 4500 with dual f.i.d. and temperature programmer). Part no. 9435 145 00021	7000
Pressure controller (part no. 9435 145 00701)	550
Flow controller (part no. 9435 145 00721)	700
Recorder (PM 8251 analytical recorder). Part no. 9443 082 51211	1720
6 x Glass columns (3 mm i.d. x 1 m length) packed with 3% OV-101 on acid-washed, silanised Chromosorb W, 80-100 mesh) each 120	720
Air compressor (PU 9003). Part no. 9423 390 03021	666
Spare parts	2000
Total	13,356

* Pye-Unicam equipment is suggested as (1) the apparatus in use in the Laboratorio Dr. Mario Muñoz was manufactured by them and (2) supply to Cuba is no problem.

C. Methods of analysis and the training of technicians

Analytical work was undertaken in the research laboratories of the Laboratorio Dr. Mario Muñoz. Samples of Agave fourcroydes juice and "coffee grounds" produced at the pilot plant at Cárdenas by Ir. Kroonen were processed and analysed by gas liquid chromatography. The staff at the Laboratorio are highly proficient in the use of this analytical technique.

High performance liquid chromatographic procedures for the analysis of steroids were discussed and research papers giving details of the methodology were given to the staff of the Laboratorio, as well as details of unpublished procedures developed in my laboratory. The need to use highly purified solvents was emphasised, but the most commonly used solvent required for the methods discussed is methanol, which should not provide any difficulty in Cuba.

D. Analytical results

Samples of Agave fourcroydes juice and "coffee grounds", prepared at the pilot plant in Cárdenas were analysed for their contents of hecogenin and tigogenin (Table 1).

Table 1. Sapogenin contents of juice and "coffee grounds" samples from the pilot plant.

Juice samples (all prepared in July, 1981)

Age of leaves used (years)	Content of sapogenin (mg/litre)		Tigogenin Content (%) (of total)
	Hecogenin	Tigogenin	
6	1128.4	190.2	14.4
7	702.6	115.1	14.0
8	897.4	164.2	15.5
unknown (mixture of ages)	1184.4	203.2	14.6
unknown (mixture of ages)	643.0	194.4	23.2

"Coffee grounds" (prepared in July, 1981)

Tigogenin content (%) of total sapogenin 23.7

Although the total sapogenin content per litre of juice is satisfactory, the proportion of tigogenin is higher than that desired (10-12%). As 6-8 year old leaves were used, which normally have low levels of tigogenin, possible variation in the relative proportions of hecogenin and tigogenin with the time of year has to be considered. Such variations occur with Agave sisalana, which is used as an economic source of hecogenin in East Africa. A study of the possible seasonal variations in the hecogenin and tigogenin proportions should be made by the Laboratorio Dr. Mario Muñoz, as it is possible that processing for hecogenin at certain times of the year may be uneconomic.

The proportion of tigogenin in the "coffee grounds" has to be carefully watched. If the ratio of tigogenin to hecogenin is too high it would probably make the sale of the "coffee grounds" impossible, or only very low prices would be obtained. If the sapogenins were extracted from the "coffee grounds", laborious crystallisation procedures would have to be used to separate the hecogenin from tigogenin. These procedures are time-consuming and result in substantial losses of hecogenin.

E. Transformation of hecogenin and other Cuban materials into steroid drugs

Chemical transformation studies are in progress on the conversion of hecogenin into final drugs, such as corticosteroids. Considerable progress has been made and many of the transformation processes have been accomplished. These studies need to be continued, with particular attention being paid to the requirements of expanding these laboratory-based experiments to the industrial scale. This "scaling-up" will involve considerable expense as several of the transformation steps require the use of high temperatures or pressures while using potentially hazardous chemicals. In particular, consideration should be given to the industrial requirements of converting hecogenin into a more highly priced intermediate, such as 11-ketotigogenin.

Several important steps in the conversion of hecogenin into corticosteroid drugs were discussed and copies of papers giving details of these processes were given to the staff at the Laboratorio.

The physical chemical equipment available at the Laboratorio for these chemical synthetic studies is not adequate for the characterisation of certain compounds made in the various reactions. As the programme continues consideration should be given to obtaining more modern equipment such as infra-red, dual beam ultraviolet and nuclear magnetic resonance spectrophotometers; all these are lacking at present.

In addition to hecogenin, other Cuban materials are being investigated as possible starting materials for conversion into steroid drugs. In particular, the sterols extracted from sugar cane waste are being studied for their suitability for transformation into contraceptive drugs. Up to 600 tons a year of total sterol can be isolated, if all the sugar cane waste were utilised, but, at present, about 2 tons a year are available from a pilot plant already erected. Several of the required chemical transformations have been achieved on the laboratory scale, but some of the early steps will require microbiological processes. It is in this area of work that outside help will be required as nobody with the necessary background is available locally. This project is of considerable interest as, with the quantity of available starting material, it should be possible to supply all of Cuba's need for contraceptive compounds.

II. RECOMMENDATIONS

1. For the effective analysis of the hecogenin and tigogenin contents of the crude sapogenin concentrates ("coffee grounds") produced at the pilot plant at Cárdenas, and in particular for the quality control of drugs, such as corticosteroids, derived from hecogenin, better analytical equipment is needed. First priority should be the purchase of high performance liquid chromatographic apparatus, which can be used for the analysis and quality control of hecogenin and all the derivatives and drugs derived from it.

2. For the routine analysis of hecogenin and tigogenin, gas-liquid chromatography is ideal. This method is being used currently at the Laboratorio Dr. Mario Muñoz, but the apparatus is an obsolete model and its expected life span is short. It is recommended that modern, replacement gas-liquid chromatographic equipment is supplied for use in conjunction with the high performance liquid chromatographic apparatus.

3. The maintenance and repair of the new equipment would be a major problem in Cuba. In addition to the original purchase of sufficient spare parts, it is necessary for EITHER a Cuban technician to be sent on a training course on the maintenance, repair and use of both high performance liquid and gas-liquid chromatographic apparatus, OR for an expert to be sent to Cuba to instal the apparatus and demonstrate the maintenance and repair of the equipment.

4. It has been recommended earlier (SI/CUB/75/001/11-03/4) that while production facilities are developed for the transformation of hecogenin into final steroid drugs, either the "coffee grounds" produced or preferably hecogenin (as hecogenin acetate) should be sold on the international market. As the current price for these commodities is depressed because of the availability of cheap hecogenin from China, consideration should be given to the conversion of hecogenin into a more highly-priced compound, such as 11-ketotigogenin, which involves only a few stages of chemical transformation.

5. The proportions of tigogenin in the samples analysed were high and so it is necessary to determine whether there is a variation in the ratio of tigogenin to hecogenin depending on the time of the year that the leaves are harvested. It is possible that at certain times of the year the ratio may be sufficiently unsatisfactory that production should be discontinued for that period.

6. Synthetic studies on the conversion of hecogenin into pharmaceutically-useful steroid drugs should be continued and consideration given to the problems of changing laboratory scale experiments to the industrial level. These transformations from laboratory to industrial scale will require considerable capital investment and technical assistance from outside the country. Moreover, for the current laboratory-based chemical studies, there is inadequate equipment for the characterisation of the products obtained. Consideration should be given, as the programme continues, to the supply of modern physical chemical equipment such as dual beam ultraviolet, infra-red and nuclear magnetic resonance spectrophotometers, all of which are absent from the Laboratorio.

7. Synthetic studies on the conversion of sugar cane sterols into contraceptive drugs should be continued. The early stages in the transformation of the sterols require microbiological processes. For this work, help will be required from outside as nobody with the necessary background is available locally in Cuba.

8. Considerable effort is being put into a programme of utilising Cuban plants related to those of known medicinal value. Several plants are being studied and, if the results are successful, indigenous materials could be used within the country instead of importing the products. As the processes involved in the isolation of these compounds are simple, "scaling up" to an industrial level should be relatively easy. Consideration should be given to assistance with this programme.

Annex 1

COUNTERPART PERSONNEL

Overall co-ordination of this project in Cuba was conducted by C.D. Daniel Calcines, Sub-director of Research, and Lic. Antonio Padilla, Manager of Natural Products Research, Vice Ministry of Pharmaceutical Industries. Other staff who co-operated in the work were Lic. Caridad Robaina, Lic. Rolando Perez, Manager of the Quality Control Department, and Lic. Alina Peña.

Annex 2

SUGGESTED TRAINING PPOGRAMME

Duration 2 months

1. Course on the assembly, use, maintenance and repair of high performance liquid chromatographic equipment.

2. Course on the assembly, use, maintenance and repair of gas-liquid chromatographic equipment.

3. Short course on the use of spectroscopic equipment - infra-red and nuclear magnetic resonance spectroscopy and mass spectrometry.