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SIMPLE COMPUTER PROGRAM FOR A LOW-COST DESK-TOP CALCULATOR APPLIED TO THE EVALUATION OF GAS—LIQUID CHROMATOGRAPHIC ANALYSES OF 17-KETOSTEROIDS AND PREGNANES*

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SUMMARY

A simple computer program consisting of 445 steps for a low-cost desk-top calculator (Hewlett-Packard 97) to be applied in chromatographic analyses of 17-ketosteroids and pregnanes from human urine samples is described. This program permits the calculation of peak factors following the chromatographic separation of an external standard mixture containing up to ten different fractions, the subsequent printout of the constants and their transcription to magnetic data cards for later retrieval when making calculations for unknown samples. After manual input of sample constants (such as total and aliquot volumes, recovery as determined by addition of a radioactive tracer steroid, internal standard) and peak factors via the data card, the individual peak heights of the samples are automatically converted to milligrams of steroid in 24-h urine and each is stored separately. All fractions can be recalled later and printed out in the order of detection or can be transformed into several diagnostically valuable parameters such as the total sum of 17-ketosteroids and pregnanes excreted, the group sums of androgens, of 11-substituted steroids and of pregnanes, the individual percentages of both the fractions and the group sums, and the ratios of aetiocholanolone—androsterone and of pregnanetriol—pregnanediol. Finally, an extension subprogram can automatically generate a plot to illustrate the steroid excretion pattern in a comprehensive fashion.

INTRODUCTION

Gas—liquid chromatography (GLC) of 17-ketosteroids (K) and pregnanes (P) is a valuable approach to the diagnosis of certain congenital enzyme deficien-

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cies, especially with respect to adrenal disorders, e.g., those involving the 11β-, the 17α- and the 21-hydroxylases which generally manifest themselves in early childhood as so-called adrenogenital syndrome with or without salt-losing syndrome. Despite the availability of specific radioimmunoassays for measuring steroid hormones and their precursors in human plasma [1—4], GLC analysis provides the important advantage of allowing the assessment of a complete steroid profile from a single sample of urine. In general, the overall procedural errors are approximately equal for all fractions analysed.

Data evaluation in GLC, however, is tedious, time consuming and prone to error, therefore necessitating the use of electronic integrators. For those laboratories which do not have access to such expensive ancillaries, we have developed a computer program that allows the rapid conversion of hand-measured peak heights into final results, expressed as milligrams of steroid in 24-h urine, as well as the output of various additional diagnostically valuable parameters (total and group sums, percentages, ratios). The hardware required is a low-cost desk-top calculator.

EXPERIMENTAL

The GLC procedure employed in our laboratory is similar to that reported by other investigators [5—8]. It involves enzymatic hydrolysis of usually one tenth of a 24-h urine sample, acidified to pH 5, using combined β-glucuronidase—steroid sulphatase (from *Helix pomatia*), diethyl ether extraction, alkaline and neutral washing, pre-purification on aluminium oxide columns, stabilization as trimethylsilyl ether derivatives, isothermal GLC fractionation on Gas-Chrom Q coated with 3% OV-225 (Supelco, Bellefonte, PA, U.S.A.) using a 2-m all-glass column in a Perkin-Elmer Model F 22 instrument and final measurement by flame ionization detection. Paper chart speed was 10 mm/min. Measurement of peak heights (h) was performed by hand.

Generally, ten different steroid fractions can clearly be separated and identified by comparison with an external standard mixture, the order of detection in our procedure being as follows: 1, pregnanediol (P2)*; 2, androsterone (A); 3, aetiocholanolone (AE); 4, dehydroepiandrosterone (D); 5, pregnanetriol (P3); 6, 11-ketoandrosterone (KA); 7, 11-ketoaetiocholanolone (KAE); 8, 11-hydroxyandrosterone (HA); 9, 11-hydroxyaetiocholanolone (HAE); and 10, pregnanetriolone (P3ON).

PROGRAM ANALYSIS

The program is written in an operational language applicable to the Hewlett-Packard 97 calculator. Algebraic formulae are expressed in Reverse Polish Nota-

*The abbreviations used represent the following systematic names: P2, 3α,20α-dihydroxy-5β-pregnane; A, 3α-hydroxy-5α-androstan-17-one; AE, 3α-hydroxy-5β-androstan-17-one; D, 3β-hydroxy-5-androsten-17-one; P3, 3α,17α,20α-trihydroxy-5β-pregnane; KA, 3α-hydroxy-5α-androstane-11,17-dione; KAE, 3α-hydroxy-5β-androstane-11,17-dione; HA, 3α,11β-dihydroxy-5α-androstan-17-one; HAE, 3α,11β-dihydroxy-5β-androstan-17-one; P3ON, 3α,17α,20α-trihydroxy-5β-pregnan-11-one; IntSt (internal standard), 5α-androstane-3,17-dione.

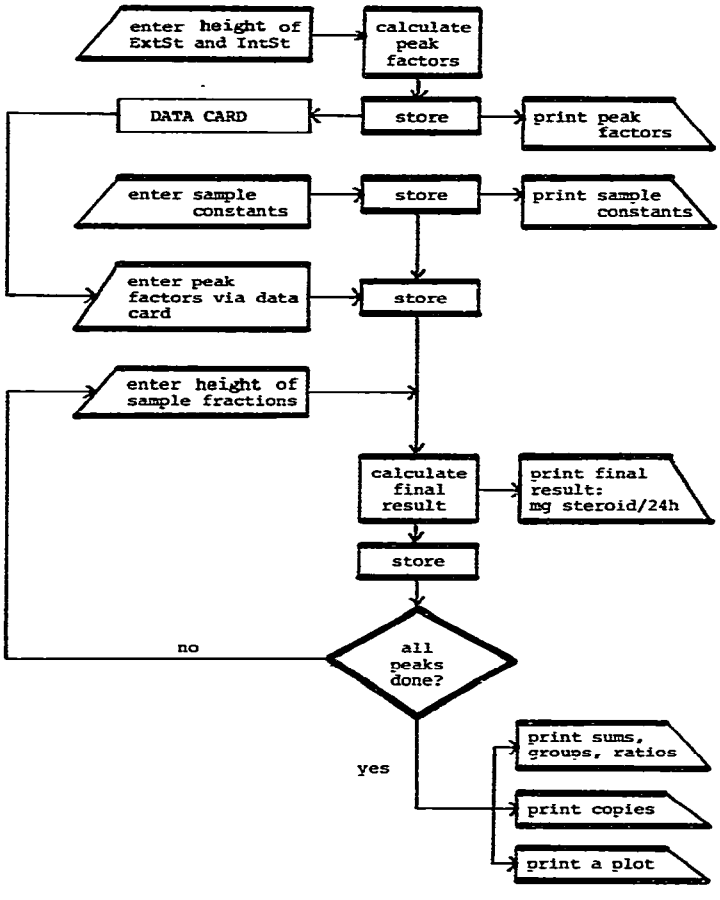

Fig. 1. Simplified flow chart representing the whole program (for abbreviations, see text).

tion. This calculator (cost less than US$ 1000) offers a 224-step memory and 25 data registers. The total program, which consists of 27 subprograms, is divided into 3 parts, each of which can be stored on a magnetic card (I, II and III). A simplified flow diagram is shown in Fig. 1 and the subprograms are listed in Table I.

Mathematics

1. The peak area (a) of a steroid fraction (S) is the product of peak height (h) and the width at half-height (w):

$$a_S = h_S w_S$$

2. The peak factor (f) of a steroid fraction contained in an external standard (ExtSt) mixture is

$$f_S = \frac{(hw)_{IntSt^*}}{(hw)_S}$$

*Internal standard within the external standard mixture. Equal amounts of each steroid fraction were injected (in our method, 1 μg).

TABLE I

LIST OF ALL SUBPROGRAMS

Letters represent those programs which are directly addressable by the operator via the corresponding keystroke; numbers stand for non-directly addressable subroutines.

Card	Label	Line	Comment
I	a	001—008	Clear all registers
	b	003—008	Clear secondary registers only
	A	009—022	Enter sample constants manually and peak factors via data card
	7	012—016	Waiting subroutine
	B	023—054	Enter sample constants (manually)
	2	025—046	Print peak factors
	4	047—054	Space formatting (4 times)
	5	049—054	Space formatting (3 times)
	1	055—098	Enter sample constants, store and print
	C	098—127	Enter *h* of steroids in external standard mixture to calculate and store on data card peak factors
	6	128—137	Print *h* of ExtSt, calculate peak factor
	D	138—166	Enter peak number, *h* of sample peaks, calculate amount excreted, sum up
	d	167—170	Space formatting (2 times)
	e	171—175	Clear sum register
	E	176—182	Print sum of steroids excreted as evaluated under subprogram D of card I
II	A	001—056	Enter *h* of all 10 sample peaks, store amount of fraction excreted in separate register
	8	057—085	Print peak number, calculate amount of steroid excreted, print
	4	086—101	Print peak number of fraction being recalled
	6	092—101	Calculate percent of steroid fraction of total ketosteroids and pregnanes
	1	102—108	Space formatting (4 times)
	3	109—181	Print total sum of steroids evaluated, sum of androgens, of 11-substituted steroids and of pregnanes; AE/A ratio, P3/P2 ratio; print percentages of fractions and groups
	5	159—181	Subroutine for register recalling
	2	182—188	Calculate percentages of fractions and groups
	B	189—200	Print copy for patient's report
	9	197—209	Subroutine for register recalling
III	C	001—035	Enter lowest and highest value of fraction excreted
	0	036—054	Plot excretion profile

3. The final amount of a steroid fraction excreted per day (mg_S/24-h urine) is:

$$\frac{(hw)_S \cdot f_S \cdot \text{aliquot factor}^{\star} \cdot 100^{\star\star}}{(hw)_{\text{IntSt}}^{\star\star\star} \cdot \dfrac{\text{recovery (\%)}}{100} \cdot 1000^{\S}}$$

*Aliquot factor = total 24-h urine volume divided by the extracted urine volume.

**If a 1/100 aliquot of total extract volume taken up in the final volume is injected (in our method generally 1/10 of total 24-h urine is extracted and, after silylation, taken up in 200 μl, of which 2 μl are injected).

***Internal standard within the unknown sample; as in the external standard, 1 μg is injected.

§ To convert μg into mg.

4. As a simplification, the product *hw* in this program is replaced by *h* alone in eqns. 2 and 3.

Algorithms

For recalling 10 different data register contents in a desired printout format, a special (program steps saving) algorithm was developed: by use of the *i*-register ("*i*" for *i*ndirect register addressing) a considerable reduction of program space was achieved. This iterative subroutine is shown in Fig. 2. The plotting algorithm has been proposed by other workers recently [9].

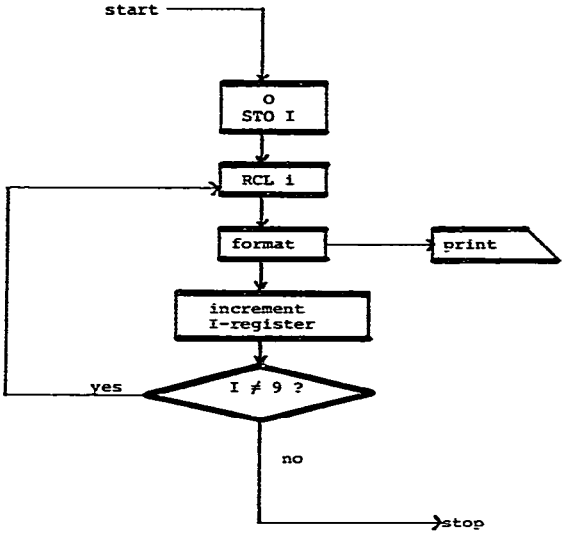

Fig. 2. Flow chart for an algorithm for recalling (RCL) primary registers No. 0 to No. 9, which contain in this program the peak factors 1—10.

PROGRAM OPERATION

The program allows the performance of the following operations in GLC data reduction.

1. Calculation of peak factors for ten different fractions from an ExtSt mixture run. After manual input of *h* of IntSt and all fractions within the ExtSt, peak factors are automatically calculated, transcribed to the magnetic data card for storage and finally printed out (see upper part of Fig. 1).
2. Calculation of selected sample fractions.
   2.1. Sample constants (24-h urine volume, extracted urine volume, radioactivity of added tracer, radioactivity recovered, *h* of IntSt within the sample) are entered manually. The previously determined peak factors are then entered via the data card and subsequently printed out for control purposes.
   2.2. The number of the peak to be evaluated (which corresponds to the order of appearance at the detector) is entered, followed by input of *h* for that peak. These data are automatically converted into the final result (mg steroid/24-h urine), printed out and summed for subsequently recalling the total sum of steroids analysed (for comparison with a

colorimetric group determination of 17-ketosteroids, e.g., the Zimmermann [10] reaction).

3. Calculation of all ten steroid fractions. Part II of the program provides an alternating order of "communication", where the calculator prints the peak number and the operator enters *h*, resulting in output of the final concentration and the number of the following peak. This sequence continues for up

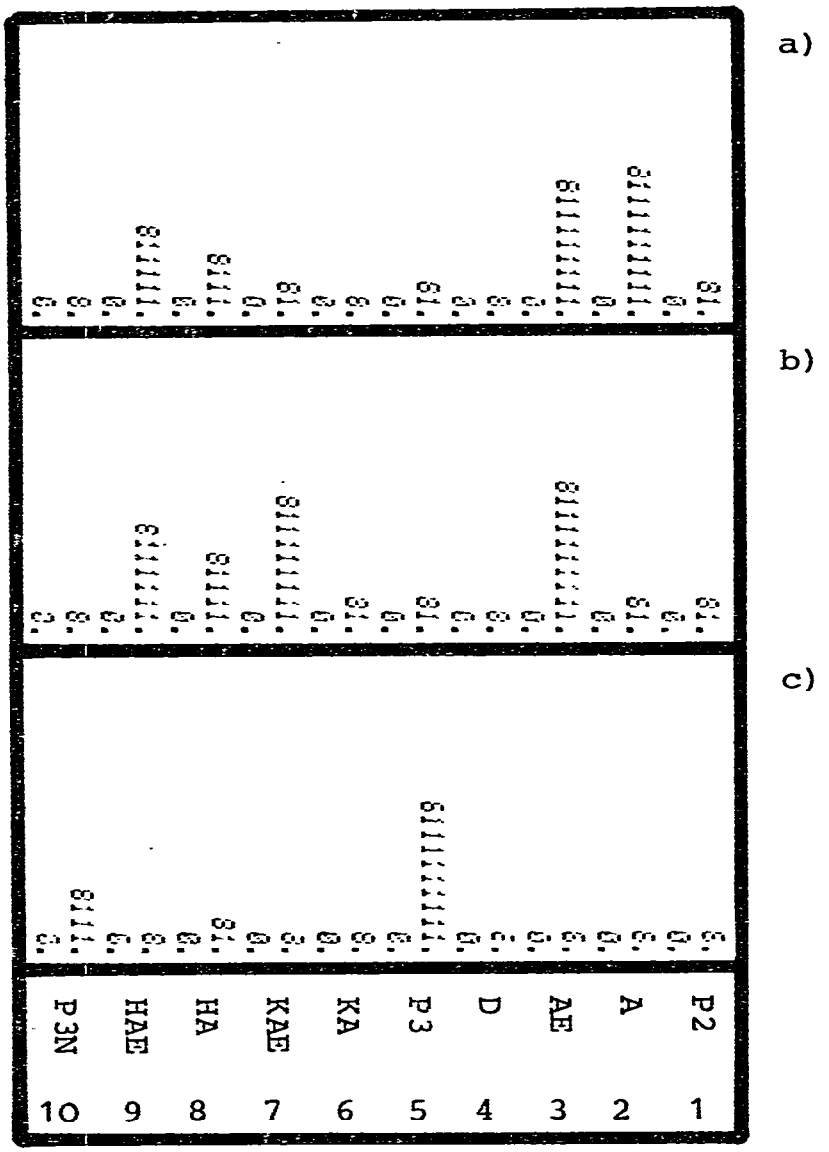

Fig. 3. Simplified computer plot to depict a steroid excretion pattern. The difference between the highest and the lowest absolute fraction value is divided into nine equal parts, the fractions being printed as multiples thereof. (a) Normal profile of a healthy 25-year-old woman in follicular phase; (b) adrenal hyperplasia due to ectopic ACTH syndrome of an adult man; (c) patient with 21-hydroxylase deficiency before treatment with dexamethasone (7-year-old girl).

to ten peaks and terminates with the printout of the following parameters:
total sum of 17-ketosteroids and pregnanes;
group sums of androgens (A, AE and D);
of 11-substituted steroids (KA, KAE, HA and HAE);
of pregnanes (P2, P3 and P3N);
the ratios of AE/A and of P3/P2; and
the percentages of all ten fractions and groups.
Further, a single keystroke produces a copy of the results to attach to the patient's report and any number of further copies thereof. Finally, a summarizing plot of the excretion pattern can be obtained by a single keystroke, which brings Part III of this program into operation (Fig. 3).

A step-by-step description of the program operation together with a listing of the program steps is given in the Appendix.

DISCUSSION

Hormone analyses require specialized methods (e.g., saturation analysis, radioimmunoassay, GLC, high-performance liquid chromatography) and, consequently, a specialized mathematical treatment of the raw data. As most endocrinological laboratories do not have extensive computer facilities, we have attempted to facilitate data reduction for a low-cost desk-top calculator (Hewlett-Packard 97) for a variety of problems, such as Scatchard plot analysis, calculation of kinetic and thermodynamic parameters of macromolecule—ligand interactions, radioimmunoassay evaluation and quality control [11—13].

The mathematics of the program presented here are based on the assumption that the signal recorded is linearly proportional to the amount of steroid injected. The range for which this is valid must be determined in each laboratory before applying this evaluation method. Otherwise, calibration graphs are necessary, from which unknown peak heights can be interpolated. Measurement of peak heights and widths at half-height is generally done visually with the aid of a magnifying lens. Provided that the peaks are approximately symmetrical, the equation for the area of a triangle (product of h and w) can be used to calculate the peak area. Alternatively, the peak height alone may be taken to relate the signal with the amount of analyte. The latter possibility is simple and straightforward and, provided the peaks are well separated from each other, very accurate. It was therefore implemented in our program, making it applicable to capillary column GLC analysis.

The automatic calculation of group sums, percentages and ratios should allow for a more efficient, clinically oriented interpretation of steroid excretion profiles. Analysis of literature data [8] and also data obtained in our laboratory suggests that, for example, the AE/A ratio is approximately 1 throughout life except in early childhood. Similarly, the P3/P2 ratio is close to 1, typical deviations occurring only during the luteal phase and pregnancy (low ratio) and in patients with 21-hydroxylase deficiency (high ratio). Androgens (A, AE and D) and 11-substituted steroids (KA, KAE, HA, HAE) generally represent together approximately 80% of the total ketosteroids and pregnanes, thus giving K : P = 4 : 1. Inverse relationships may be found in adrenogenital syndrome where, additionally, a high P3/P2 ratio and the presence of P3ON (pregnanetriolone)

is pathognomonic for 21-hydroxylase deficiency, whereas low levels or the absence of 11-substituted steroids are indicative of the 11β-type enzyme deficiency. High levels of androgens can be observed in some patients with idiopathic hirsutism [14], sometimes associated with an atypically high absolute or percentage value for dehydroepiandrosterone.

Hence, the calculation subprogram described here produces a number of diagnostically valuable parameters, facilitating interpretation and allowing immediate recognition of abnormal or pathological levels in a gas—liquid chromatogram of fractionated 17-ketosteroids and pregnanes. Further, this program could easily be modified for a variety of other chromatographic problems. After 2 years' experience, we are able to present this program as a realistic alternative to an expensive chromatographic profile integrator module.

ACKNOWLEDGEMENTS

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APPENDIX

Description of program operation

General

(a) Switch the calculator into operation mode "PRGM" (for program editing). Key in the program steps of Part I, as listed in Table V. After having entered all steps, insert an empty magnetic card for permanent storage of the program. Clip the card on both edges to protect of erasing. Parts II and III are programmed in the same way.
(b) Switch the calculator into operation mode "RUN" (for program operation).

1. Calculation of peak factors

1.1. Use card I, calculator being in the "RUN" position. Press /a/ for initializing (= for clearing all register contents).
1.2. Enter height (h) of IntSt in external standard mixture, press /C/.
1.3. Enter h of $ExtSt_1$, press /R/S/.
1.4. Repeat step 1.3 for $ExtSt_{2-10}$.
1.5. After having entered h of $ExtSt_{10}$ and having pressed /R/S/, "Crd" will appear on the display. Following the insertion of an empty magnetic data card reserved for that purpose only, peak factors will be permanently stored on this data carrier. An automatic printout of peak factors thus calculated terminates the program.
1.6. Attach outprint as proposed in Table II.

2. Calculation of selected sample steroid fractions

2.1. When starting the calculations, repeat step 1.1.

2.2.1. Enter 24-h urine volume (ml), press /A/.

2.2.2. Enter extracted urine volume (ml), press /R/S/.

2.2.3. Enter total counts of recovery tracer added, press /R/S/.

2.2.4. Enter counts of recovery tracer found, press /R/S/.

2.2.5. Enter *h* of IntSt in sample, press /R/S/.

2.2.6. Press /R/S/ and immediately insert data card (holding the previously determined peak factors). After a short pause (effected by a special waiting routine within the program), the data card will automatically pass the reading station; 9.000000000 will appear in the display for a few seconds and then the printout of peak factors will signal to the operator the end of this program.

2.3. For calculation of a further sample on the basis of the same peak factors, step 2.2.6 can be omitted and step 2.2.1 is changed by pressing /B/ instead of /A/. In this case, peak factors will be printed out for control automatically without inserting the data card.

2.4. Attach printout.

2.5.1. Enter number of the peak to be evaluated (the number corresponds to the order of appearance at the detection unit as assessed by the ExtSt mixture), press /D/.

2.5.2. Enter *h* of this peak, press /R/S/: mg steroid/24-h urine will be printed out.

2.6. Repeat steps 2.5.1 and 2.5.2 for all further peaks; press /d/ once when omitting one peak (correspondingly, press /d/ twice when leaving out 2 peaks).

2.7. If a sum of all peaks calculated is desired, press /E/.

2.8. For clearing this sum register, press /e/.

TABLE II

PROTOCOL AND PRINTOUT FOR CALCULATING PEAK FACTORS FROM AN EXTERNAL STANDARD MIXTURE GLC RUN, AND PROTOCOL OF SAMPLE CONSTANTS

Sample constants: 24-h urine volume, 900 ml; incubated volume, 100 ml; recovery, 65% (total cpm added, 10.000; cpm recovered, 6.500); internal standard *h*, 23.0

Peak No.	Fractionated 17-ketosteroids and pregnanes		Height (mm)	Peak factor
Internal standard		—	37.5	—
1	P2	Pregnanediol	182.0	0.206
2	A	Androsterone	189.0	0.198
3	AE	Aetiocholanolone	143.5	0.261
4	D	Dehydroepiandrosterone	211.0	0.178
5	P3	Pregnanetriol	106.0	0.354
6	KA	11-Ketoandrosterone	83.0	0.452
7	KAE	11-Ketoaetiocholanolone	82.0	0.457
8	HA	11-Hydroxyandrosterone	59.0	0.636
9	HAE	11-Hydroxyaetiocholanolone	51.5	0.728
10	P3ON	Pregnanetriolone	43.1	0.870

TABLE III

PROTOCOL AND PRINTOUT OF SAMPLE FRACTIONS, TOGETHER WITH SUMS, PERCENTAGES OF FRACTIONS AND OF GROUPS, AND RATIOS APPLYING THE SAMPLE CONSTANTS AND PEAK FACTORS OF TABLE II

Peak No.	Sample peak	Peak height (mm)	mg/24 h	%
1	P2	22	0.273	3.5
2	A	202	2.413	31.3
3	AE	152	2.391	31.1
4	D	2	0.021	0.3
5	P3	19	0.405	5.3
6	KA	4	0.109	1.4
7	KAE	11	0.303	3.9
8	HA	18	0.689	8.9
9	HAE	25	1.096	14.2
10	P3ON	0	0.000	0.0
Total sum of K and P			7.70	
Androgens			4.83	62.7
11-Substituted K			2.20	28.5
Pregnanes			0.68	8.8
AE/A ratio			0.99	
P3/P2 ratio			1.48	

TABLE IV

PATIENT'S REPORT PRINTOUT AS CALCULATED IN THE PREVIOUS PROGRAM SECTION (SEE TABLE III)

Peak No.	Component	mg/24 h
1	P2	0.273
2	A	2.413
3	AE	2.391
4	D	0.021
5	P3	0.405
6	KA	0.109
7	KAE	0.303
8	HA	0.689
9	HAE	1.096
10	P3ON	0.000

3. Calculation of all ten steroid fractions

3.1. Instead of doing step 2.5.1, insert program card II and press /A/; number "1" will be printed out to ask for h of the 1st peak.

3.2. Enter h_1, press /R/S/: result in mg steroid/24-h urine will be printed out together with number "2".

3.3. Enter h_2 and so on for all ten peaks.

3.4. This sequence will, after the printout of the last peak's result, automatically produce the following further outputs: total sum, groups sums and ratios. Cut off printout and press /R/S/: the percentage of all fractions and groups will be printed out. Cut off printout and attach to protocol sheet as proposed in Table III.

TABLE V

INDIVIDUAL PROGRAM STEPS OF PARTS I AND III

Card I

001	*LBLa	060	PRTX	119	STO8	178	RCLE
002	CLRG	061	STOB	120	R/S	179	DSP2
003	*LBLb	062	RCLA	121	GSB6	180	PRTX
004	P≠S	063	RCLB	122	STO9	181	GSB4
005	CLRG	064	÷	123	SPC	182	RTN
006	P≠S	065	STOA	124	SPC		
007	CLX	066	SPC	125	WDTA		
008	RTN	067	R/S	126	GSB2		
009	*LBLA	068	STOB	127	RTN		
010	GSB1	069	R/S	128	*LBL6		
011	R/S	070	RCLB	129	DSP1		
012	5	071	÷	130	STOI		
013	STOI	072	EEX	131	PRTX		
014	*LBL7	073	2	132	RCLA		
015	DSZI	074	x	133	RCLI		
016	GTO7	075	DSP1	134	÷		
017	9	076	PRTX	135	DSP3		
018	STOI	077	STOB	136	RTN		
019	MRG	078	SPC	137	*LBLD		
020	PSE	079	R/S	138	DSPØ		
021	GSB2	080	PRTX	139	PRTX		
022	RTN	081	STOC	140	1		
023	*LBLB	082	SPC	141	−		
024	GSB1	083	SPC	142	STOI		
025	*LBL2	084	CLX	143	R/S		
026	DSP3	085	DSP9	144	DSP1		
027	RCLØ	086	RTN	145	STOD		
028	PRTX	087	*LBLC	146	RCLC		
029	RCL1	088	DSP1	147	÷		
030	PRTX	089	PRTX	148	RCLi		
031	RCL2	090	STOA	149	x		
032	PRTX	091	SPC	150	RCLA		
033	RCL3	092	SPC	151	x		
034	PRTX	093	R/S	152	RCLB		
035	RCL4	094	GSB6	153	EEX		
036	PRTX	095	STOØ	154	1		
037	RCL5	096	R/S	155	÷		
038	PRTX	097	GSB6	156	÷		
039	RCL6	098	STO1	157	DSP2		
040	PRTX	099	R/S	158	PRTX		
041	RCL7	100	GSB6	159	STOI		
042	PRTX	101	STO2	160	RCLE		
043	RCL8	102	R/S	161	RCLI		
044	PRTX	103	GSB6	162	+		
045	RCL9	104	STO3	163	STOE		
046	PRTX	105	R/S	164	RCLI		
047	*LBL4	106	GSB6	165	DSP1		
048	SPC	107	STO4	166	RTN		
049	*LBL5	108	R/S	167	*LBLd		
050	SPC	109	GSB6	168	SPC		
051	SPC	110	STO5	169	SPC		
052	SPC	111	R/S	170	RTN		
053	CLX	112	GSB6	171	*LBLe		
054	RTN	113	STO6	172	Ø		
055	*LBL1	114	R/S	173	STOE		
056	DSPØ	115	GSB6	174	RCLE		
057	PRTX	116	STO7	175	RTN		
058	STOA	117	R/S	176	*LBLE		
059	R/S	118	GSB6	177	GSB5		

Card III

001	*LBLC
002	STOA
003	X≠Y
004	−
005	9
006	÷
007	STOB
008	1
009	Ø
010	STOI
011	RCLi
012	GSBØ
013	*LBL7
014	ISZI
015	RCLi
016	GSBØ
017	1
018	9
019	ENT↑
020	RCLI
021	X≠Y?
022	GTO7
023	Ø
024	STOA
025	STOB
026	STOC
027	STOD
028	STOE
029	SPC
030	SPC
031	SPC
032	SPC
033	CLX
034	DSP9
035	RTN
036	*LBLØ
037	RCLA
038	−
039	DSPØ
040	RCLB
041	÷
042	9
043	+
044	RND
045	10^x
046	9
047	1/X
048	8
049	+
050	x
051	PRTX
052	Ø
053	PRTX
054	RTN

TABLE VI
INDIVIDUAL PROGRAM STEPS OF PART II

Card II

001	*LBLA	060	1	119	RCL2	178	GSB2
002	Ø	061	−	120	+	179	PRTX
003	STOE	062	STOI	121	RCL3	180	GSB1
004	1	063	R/S	122	+	181	RTN
005	GSB8	064	DSP1	123	STOA	182	*LBL2
006	P≠S	065	STOD	124	PRTX	183	RCLE
007	STOØ	066	RCLC	125	RCL5	184	÷
008	P≠S	067	÷	126	RCL6	185	EEX
009	2	068	RCLi	127	+	186	2
010	GSB8	069	x	128	RCL7	187	x
011	P≠S	070	RCLA	129	+	188	RTN
012	STO1	071	x	130	RCL8	189	*LBLB
013	P≠S	072	RCLB	131	+	190	1
014	3	073	EEX	132	STOB	191	Ø
015	GSB8	074	1	133	PRTX	192	STOI
016	P≠S	075	÷	134	RCLØ	193	SPC
017	STO2	076	÷	135	RCL4	194	RCLi
018	P≠S	077	DSP3	136	+	195	DSP3
019	4	078	PRTX	137	RCL9	196	PRTX
020	GSB8	079	STOI	138	+	197	*LBL9
021	P≠S	080	RCLE	139	STOC	198	ISZI
022	STO3	081	RCLI	140	PRTX	199	SPC
023	P≠S	082	+	141	SPC	200	RCLi
024	5	083	STOE	142	RCL2	201	PRTX
025	GSB8	084	RCLI	143	RCL1	202	1
026	P≠S	085	RTN	144	÷	203	9
027	STO4	086	*LBL4	145	DSP2	204	ENT↑
028	P≠S	087	RCLI	146	PRTX	205	RCLI
029	6	088	9	147	SPC	206	X≠Y?
030	GSB8	089	−	148	RCL4	207	GTO9
031	P≠S	090	DSPØ	149	RCLØ	208	GSB1
032	STO5	091	PRTX	150	P=S	209	RTN
033	P≠S	092	*LBL6	151	÷		
034	7	093	RCLi	152	PRTX		
035	GSB8	094	RCLE	153	GSB1		
036	P≠S	095	÷	154	R/S		
037	STO6	096	EEX	155	1		
038	P≠S	097	2	156	Ø		
039	8	098	x	157	STOI		
040	GSB8	099	DSP1	158	GSB4		
041	P≠S	100	PRTX	159	*LBL5		
042	STO7	101	RTN	160	ISZI		
043	P≠S	102	*LBL1	161	GSB4		
044	9	103	SPC	162	1		
045	GSB8	104	SPC	163	9		
046	P≠S	105	SPC	164	ENT↑		
047	STO8	106	SPC	165	RCLI		
048	P≠S	107	CLX	166	X≠Y?		
049	1	108	RTN	167	GTO5		
050	Ø	109	*LBL3	168	GSB1		
051	GSB8	110	SPC	169	SPC		
052	P≠S	111	SPC	170	RCLA		
053	STO9	112	SPC	171	GSB2		
054	P≠S	113	RCLE	172	DSP1		
055	GSB3	114	DSP2	173	PRTX		
056	RTN	115	PRTX	174	RCLB		
057	*LBL8	116	SPC	175	GSB2		
058	DSPØ	117	P≠S	176	PRTX		
059	PRTX	118	RCL1	177	RCLC		

3.5. If a copy of the result is desired, press /B/. Repeat this step if necessary.
3.6. If a plot of the excretion pattern is desired, insert program card III, enter the lowest value (mg/24 h), press /ENTER↑/, enter highest one, press /C/ and the plot will be automatically generated.

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