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by

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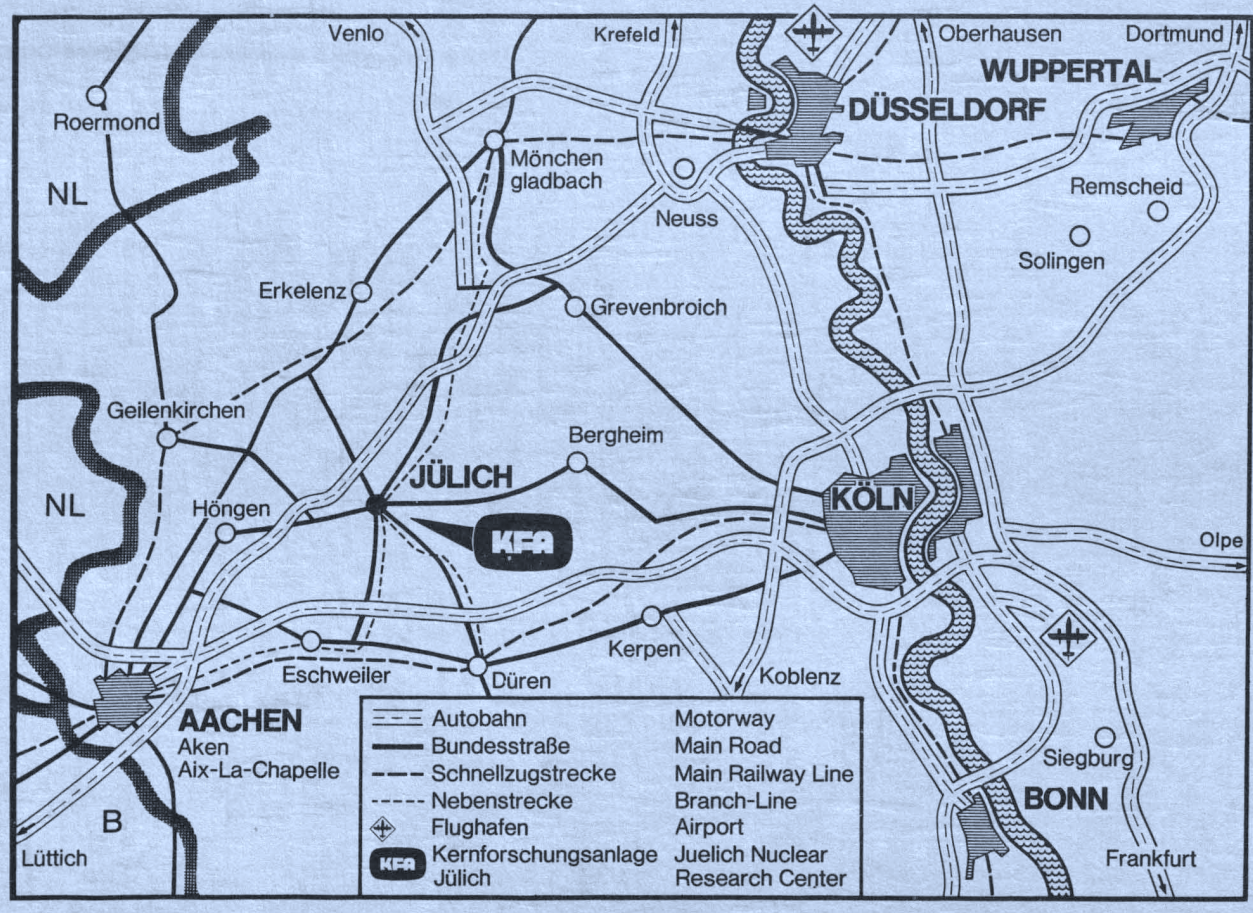

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Contents

Summary

This report is intended to serve as a practical guide, elaborately describing the working details and some developmental work, connected to an automated multielement radiochemical machine based on ion-exchange and partition chromatography. Some of the practical aspects and personal observations after much experience with this versatile multi-element method, applied to investigate the elemental composition of different biomedical matrices, are summarized. Standard reference materials (SRM) are analyzed, and the data are presented with a set of gamma-spectra, obtained before and after chemical separation into convenient groups suitable for gamma spectrometry. The samples analyzed included various human and animal tissues, body-fluids, IAEA biological standard reference materials, and samples from the WHO/IAEA project on "Trace elements in relation to cardio-vascular diseases". Simplified modifications of the radio-chemical processing, suitable for fast and routine analysis of clinical samples have also been discussed.

1. Fundamental requirement of a good multielement radiochemical scheme.

The need for radiochemical separations in multielement NAA of human tissues and body-fluids has been exclusively discussed by PARR(1). In this connection, a number of procedures have been discussed and among them, two methods, one based on solvent extraction developed by TROWELL et al.(1, p.133), and the other based on ion-exchange chromatography developed by SAMSAHL et al.(1, P. 155), were found to be especially attractive owing to the automation. This report exclusively describes the procedural and developmental aspect of the ion-exchange chromatographic method, which has been used in our laboratory, to analyse samples for the WHO/IAEA project.

Several radiochemical multielement methods for processing neutron irradiated biological samples, based on ion-exchange chromatography have been developed. Even with automated systems, following sorption, if elution procedures are involved, when many elements requiring further separation are adsorbed on a single column, this practice is not always practical for a large number of samples. Comprehensive group separations, which permit direct measurement of the columns based only on sorption, thus greatly minimising the post-sorption handling required to resolve the selectively grouped elements, using either the Na(I) or the Ge(Li) system, are not many. One such scheme developed by SAMSAHL advantageously combines a series of ion-exchange and partition chromatographic columns, resulting in the automated set-up SA-7100 (AB Atomenergi, Sweden). The basis of this scheme is,

a) primary separation of the volatile elements from the non-volatile elements,
b) fixed-reproducible yield through selective sorption at higher flow rate for further sub-grouping, and
c) use of the monitor factor (MF) to avoid repeated irradiation of the standards and application of the pre-determined geometry factors (GF), for the simultaneous correction of the recovery in the chemical processing and the geometry differences in the counting operations.

Besides speed, flexibility is seen in the possibility to shorten the scheme to suit the determination of a few selected number of ele-

ments, without too many manipulations. Since the various chemical conditions constituting the entire separation procedure are accomplished through an automated working system, despite the fact that, many chemical manipulations are involved to obtain an interference-free sub-grouping of the desired elements, if the machine is maintained carefully, the reproducibility of the separation is good. Another attractive aspect of this set-up is the provision to incorporate developmental changes, specific to a problem at hand, maintaining the basic features essentially unchanged.

2. Preparation of the stock standard solutions.

Table 1 shows the composition of various standard solutions. All the chemicals are of p.a. grade and double distilled water is used to prepare the solutions. For certain elements 'Titrisol' standards can be used with appropriate dilution, thus saving much time.

Mixed standards: Following combinations are used, as they occur in selected groups following chemical separation, excepting for the set mentioned under item 9.

1. Au + Mo + W
2. Ag + Cd
3. Fe + Zn
4. Ca + Sc
5. Ce + La
6. Co + Cr + Cu
7. Cs + K + Na + Rb + P
8. As + Se
9. Br, Hg, Mn, Sb and Sn are prepared as single standards.

In most cases, the concentrations of the mixed standards are so adjusted that by pipetting 50 µl, desired quantity of the elements is obtained. These are pipetted into cleaned (4 mm internal diameter and 5 cm long) quartz tubes and dried at 50°C. Then they are sealed under water cooling.

Carrier solutions: Carrier solution-1 is prepared by pipetting the various stock standard solutions as mentioned. Composition is shown in Table 1. The mixture is finally made up to 50 ml using concentrated HNO_3. The final concentration will be 1 µg in 50 µl

Table 1

	Element	Chemical form	Quantity of the element in mg per 100 µl	Amount of the Compound dissolved in 250 ml		Dissolution medium
1	Ag	$AgNO_3$	0.1	393.70	mg	4N NH_4OH
2	As	As_2O_3	0.01	33.01	mg	"
3	Au	Au 23K	$2 \cdot 10^{-4}$	0.5	mg	0.5N HNO_3
4	Ba	$Ba(NO_3)_2$	2.0	9.51471	g	"
5	Br	NH_4Br	0.01	30.64	mg	4N HN_4OH
6	Ca	$CaCO_3$	10.0	62.43138	g	6N HCl
7	Cd	$Cd(NO_3)_2.4H_2O$	0.1	686.13	mg	0.5N HNO_3
8	Ce	$CeCl_3.7\ H_2O$	0.5	3.32286	g	"
9	Cl	NH_4Cl	0.01	37.72	mg	4N NH_4OH
10	Co	$CoCl_2\ 6\ H_2O$	0.05	504.69	mg	0.5N HNO_3
11	Cr	$CrCl_3.6\ H_2O$	0.1	1.28066	g	"
12	Cs	CsCl	0.05	158.32	mg	H_2O
13	Cu	$CuCl_2.2\ H_2O$	0.05	335.36	mg	0.5N HNO_3
14	Fe	$Fe(NO_3)_3.9\ H_2O$	5.0	90.4252	g	"
15	Ga	Ga-metal	0.05	125.01	mg	"
16	Hg	$HgSO_4$	0.1	369.71	mg	2N H_2SO_4
17	In	In-metal	0.05	125.00	mg	0.5N HNO_3
18	I	NH_4I	0.1	285.54	mg	4N NH_4OH
19	K	K_2CO_3	5.0	22.0924	g	H_2O
20	La	$La(NO_3)_3 6\ H_2O$	0.01	77.92	mg	0.5N HNO_3
21	Mg	$MgCl_2.6\ H_2O$	5.0	104.5403	g	H_2O
22	Mn	$MnSO_4.4\ H_2O$	0.01	101.47	mg	0.5N HNO_3
23	Mo	MoO_3	0.1	375.09	mg	4N NH_4OH
24	Na	NaCl	0.5	3.17804	g	H_2O
25	Ni	$Ni(NO_3)_2.6\ H_2O$	5.0	61.91662	g	0.5N HNO_3
26	P	$NH_4H_2PO_4$	1.0	9.28559	g	0.1N HNO_3
27	Rb	RbCl	0.5	1.76854	g	H_2O
28	Sb	Sb_2O_3	0.01	29.93	mg	6N HCl
29	Sc*	Sc_2O_3	0.05	191.71	mg	0.5N HNO_3
30	Se	SeO_2	0.2	702.64	mg	4N NH_4OH
31	Sn	$SnCl_2.2\ H_2O$	1.0	4.75250	g	1N HCl
32	Sr	$Sr(NO_3)_2$	0.5	3.01910	g	0.5N HNO_3
33	W	$WO_3 \cdot H_2O$	0.01	33.98	mg	4N NH_4OH
34	Zn	Zn-metal	1.0	2.50004	g	0.5N HNO_3

*To dissolve Sc: Dissolve the 191.71 mg of Sc_2O_3 in 5 ml of conc. HCl, adding 30% H_2O_2 drop-wise while boiling the solution in a 150 ml tall beaker covered with watch glass. Following dissolution dilute the residue with 0.5N HNO_3 to 250 ml.

for all the elements, excepting Au, which will be 10 ng in 50 µl.

Carrier solution-1

Ag^{+} = 1 ml	Fe^{3+} = 20 µl	
Au^{3+} = 5 ml	La^{3+} = 10 ml	
Ca^{2+} =25 µl	Mn^{2+} = 10 ml	
Cd^{2+} = 1 ml	Mo^{6+} = 1 ml	
Ce^{3+} =200µl	Rb^{+} =200 µl	
Co^{2+} = 2 ml	Sc^{3+} = 2 ml	
Cr^{3+} = 1 ml	W^{6+} = 10 ml	
Cs^{+} = 2 ml	Zn^{2+} =100 µl	
Cu^{2+} = 2 ml		

Carrier solution-2

61 mg As_2O_3, 119,7 mg Sb_2O_3, 73,94 mg $HgSO_4$ and 95 mg $SnCl_2.2H_2O$ are individually dissolved and made up to 50 ml volume with 48 % HBr. Final concentration will be 50 µg/50 µl of each of the four elements.

Carrier solution-3

1686 mg of SeO_2 is dissolved in 20 ml of water to give 3 mg Se/50 µl.

3. Preparation of the stock process solutions

Non-volatile group:

Following solutions are prepared for this group.

1. 0.4-N HBr
   45 ml 48% HBr is diluted to 1 litre.

2. 11.3-N LiCl solution
   479 g LiCl is dissolved in 1 litre of water. Some heating is necessary.

3. 4-N NaAc solution
   328.2 g NaAc is dissolved in 1 litre of water. Some heating is necessary.

4. 10-N NaOH
400 g NaOH is dissolved in water and diluted to 1 litre.

5. NaAc-NaOH solution
600 ml 4-N NaAc, 200 ml 10-N NaOH and 90 ml distilled water are mixed.

6. Washing solution 1

84 ml conc H_2SO_4, 200 ml 1.5-M Na_2SO_4 and 20 ml 30 % H_2O_2 are diluted to 2000 ml with distilled water.

(1.5-M Na_2SO_4 is prepared by dissolving 241.6 g $Na_2SO_4.10H_2O$ in distilled water and diluted to 500 ml. Some heating may be necessary to bring the salt into solution. Anhydric Na_2SO_4 has been tried but has sometimes been difficult to dissolve completely).

This is used for washing column 1.

7. Washing solution 2
8 parts of washing solution 1 and 1 part of 0.4-N HBr are mixed.

This is used for washing column 2.

8. Washing solution 3
8 parts of washing solution1, 1 part of 0.4-N HBr and 4 parts of 11.3-N LiCl are mixed.

This is used for washing columns 3 and 4.

9. Washing solution 4
8 parts of washing solution 1, 1 part of 0.4-N HBr, 4 parts of 11.3-N LiCl and 4 parts of NaAc-NaOH solution (ref.5) are mixed.

This is used for washing the columns 5 and 6.

Following solutions are used during the sample preparation:

10. 0.3 % H_2O_2

1 part of 30 % H_2O_2 is diluted with 100 parts of distilled water.

11. 3-N NaCl
175.4 g NaCl is dissolved and diluted to 1 litre with distilled water.

In general, pro analysi chemicals shall be used. It is advisable to prepare large quantities.

Volatile group:

Wash solution 5: 216 ml of water. 20 ml 97 % H_2SO_4, 160 ml 12 N HCl and 4 ml 30 % H_2O_2 is mixed in the same order as mentioned taking care to cool the solution after H_2SO_4 addition to avoid vigorous gas formation during HCl-H_2O_2 addition.

This is used for washing column 9.

Solution-6: 150 ml of 48 % HBr p.a. is mixed with 300 ml of 9 N LiBr. It may be necessary to filter the LiBr solution before use.

Wash solution-6: 1 partof solution 6 is mixed with one part of wash solution 5.

This is used to wash column 10.

4. Preparation of the ion-exchange and chromatographic columns for the chemical separation.

Column details:

Column number	Column dimensions	Column material and ionic-form	elements adsorbed
1	10x25 mm	Bio-Rad Ag 2x8 HSO_4^-, 200-400 mesh	Au,Mo,W
2	10x15 mm	Bio-Rad Ag 2x8 HSO_4^-, 200-400 mesh	Ag,Cd
3	14x25 mm	Bio-Rad Ag 1x4 Cl^-, 200-400 mesh	Fe,Zn
4	10x15 mm	HDEHP treated Kiselguhr	Ca,Sc
5	10x15 mm	HDEHP treated Kiselguhr	Ce,La
6	14x25 mm	Bio-Rad Chelex, 100 Na^+, 200-400 mesh	Co,Cr,Cu,Mn
7	14x25 mm	Bio-Rad Chelex 100 Al^{3+}, 200-400 mesh	P
8	14x45 mm	Bio-Rad ZP-1 50-100 mesh	Cs,K,Rb
9	10x25 mm	Bio-Rad Ag 2x8 Cl^-, 200-400 mesh	Hg,Sb,Sn
10	14x45 mm	Bio-Rad Ag 2x8 Cl^-, 200-400 mesh	As,Se

Columns 7 and 8 are not generally necessary, and the effluent leaving the column 6 can be collected directly. The determination of P has some difficulties (see page 22). Na and K can be measured soon after separation. Cs and Rb can be conveniently measured following the decay of ^{24}Na and ^{42}K.

Pre-treatment: It is important to strictly isolate the different column materials from each other till the columns are prepared and secured between the filters safely. The particles from one column material in another column interfere with the recovery values, e.g. particles from chelex resin, if present in the earlier columns of the series will result in complexing certain elements on wrong columns. Similarly, traces of HDEHP too can interfere.

About 300 g of AG 2x8, 200 g each of AG 1x4 and Chelex-100 ion-exchangers are separately taken in 1 litre capacity measuring cylinders with stopper, and are rigorously shaken with 4-5 hundred ml of distilled water. After necessary settling time the water layer with dust-like particles is decanted. This treatment with water is repeated two times more as it is of utmost practical importance to remove the colloidal particles completely, to avoid higher resistance to the influent solution in each column, during the separation. After the final washing the ion-exchangers are individually filtered on a funnel and sucked dry. Further treatment is as follows:

a) 200 g of the AG 2x8 resin is stored under water without any further treatment and <u>is used for the columns 9 and 10</u>.

b) The remaining batch of 100 g of the AG 2x8 resin is retained on the funnel, washed twice with 100 ml of wash-solution 1 and sucked dry. Finally, the resin is taken in a beaker, stirred well with 150 ml of wash-solution-1 and stored in polyethelene bottles.

This resin is used for columns 1 and 2.

c) Similarly the resin Ag 1x4 is also filtered off and is treated twice with 200 ml of wash-solution 3, and then stored under 300 ml of the same wash solution.

This resin is used for column 3.

d) Chelex-100 is also handled in the same manner, using wash-solution 4 for washing and storing.

This resin is used for column 6.

Storing of the ion-exchange resins in solutions corresponding to the influent flow during the separation step is necessary, to establish the equilibrium between the resin and the solution phase and importantly, to avoid the risk of swelling and shrinking of the organic ion-exchangers exposed to different ionic strength and pH conditions.

Preparation of HDEHP treated Kiselguhr: For the partition chromatographic columns celite-545 (Johns-Manville) is used as the solid support. About 100 g of celite-545 is taken in a beaker and washed with distilled water a number of times to completely remove all the fine particles. After final washing, it is sucked

dry on a funnel and washed once with acetone. It is then thoroughly dried at 110°C for a few hours, occasionally stirring the contents. The dry powder is taken on a filter and 10 ml of dimethylchlorsilan is poured on top of the celite powder and allowed to stand for 2 hours. If moisture is present, dimethylchlorsilan reacts violently. Generally by this interval the celite will have been siliconized. The siliconized celite is then sucked dry, mixed well and stored in a polyethelene bottle.

15 g of the siliconized celite is taken in a small beaker and 1.5 g of HDEHP [di-(2-ethylhexyl)orthophosphoric acid] dissolved in 50 ml of isopropylether, is added and thoroughly stirred. The contents are left over night in a fume-hood to evaporate the ether. The residue is taken in 50 to 100 ml of distilled water, stirred well and preserved in polyethelene bottle.

This material is used for columns 4 and 5.

Preparation and storing of the columns: The columns are prepared using a suction flask. Precision in preparing the columns is of utmost importance. If over filled and subsequently tightly pressed in, it will result in building up pressure during separation eventually creating leaks and ending up in contamination hazards, among other things. Loose and insufficient packing on the other hand, may affect recovery values. It is a good practice to test the flow through the columns, soon after preparing them, using corresponding wash solutions. These columns may be prepared in large numbers at a time. If stored in a desiccator with little water in it, the columns will remain moist for months.

5. Sample dissolution, distillation and radiochemical separation using SA-7100.

The basic chemical concepts of this automated set-up is the result of the systematic improvement of a rather extensive and elaborate separation scheme developed over a number of years (2,3,4,). Information derived by a series of experiments in different

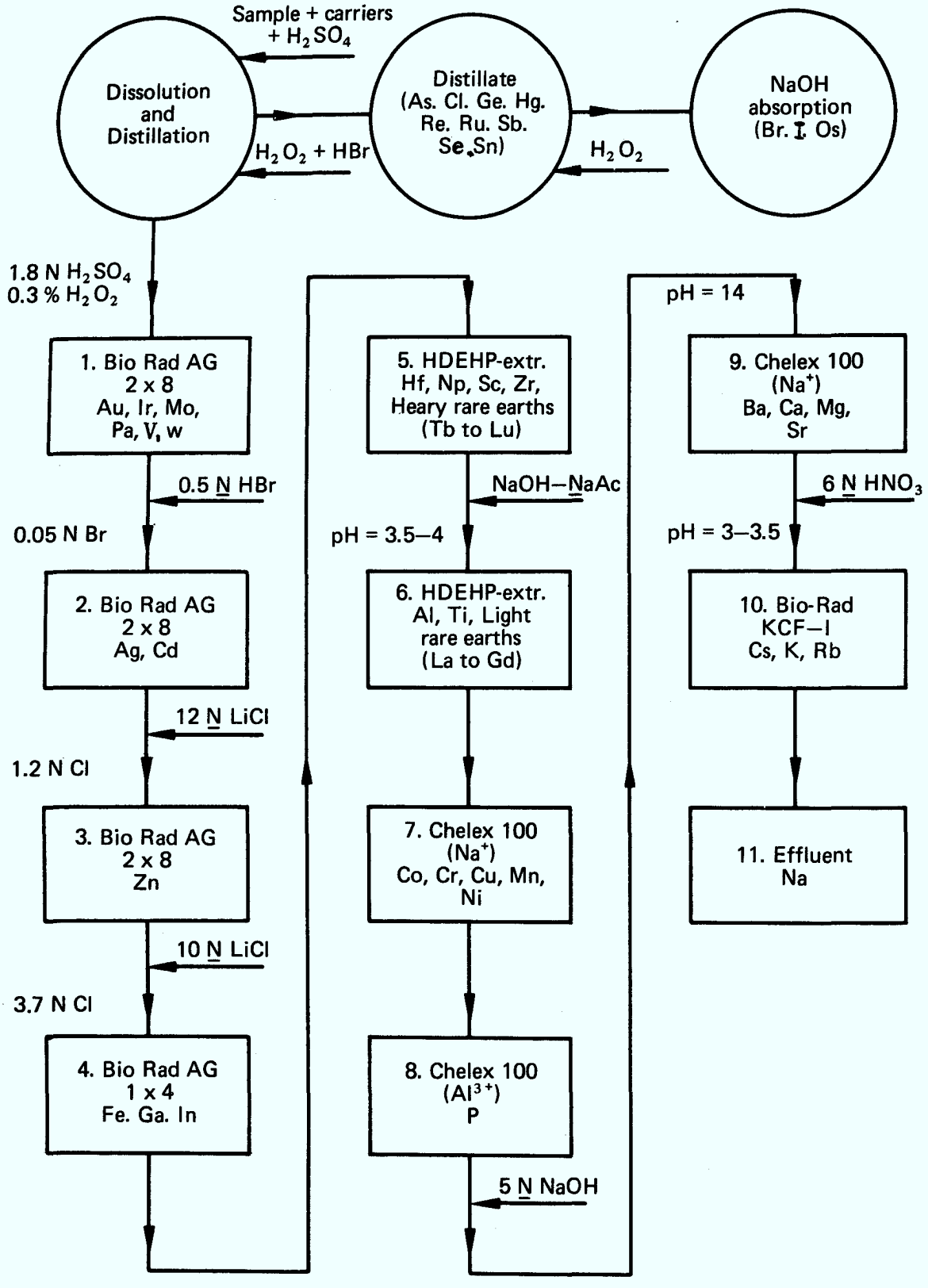


Figure 1

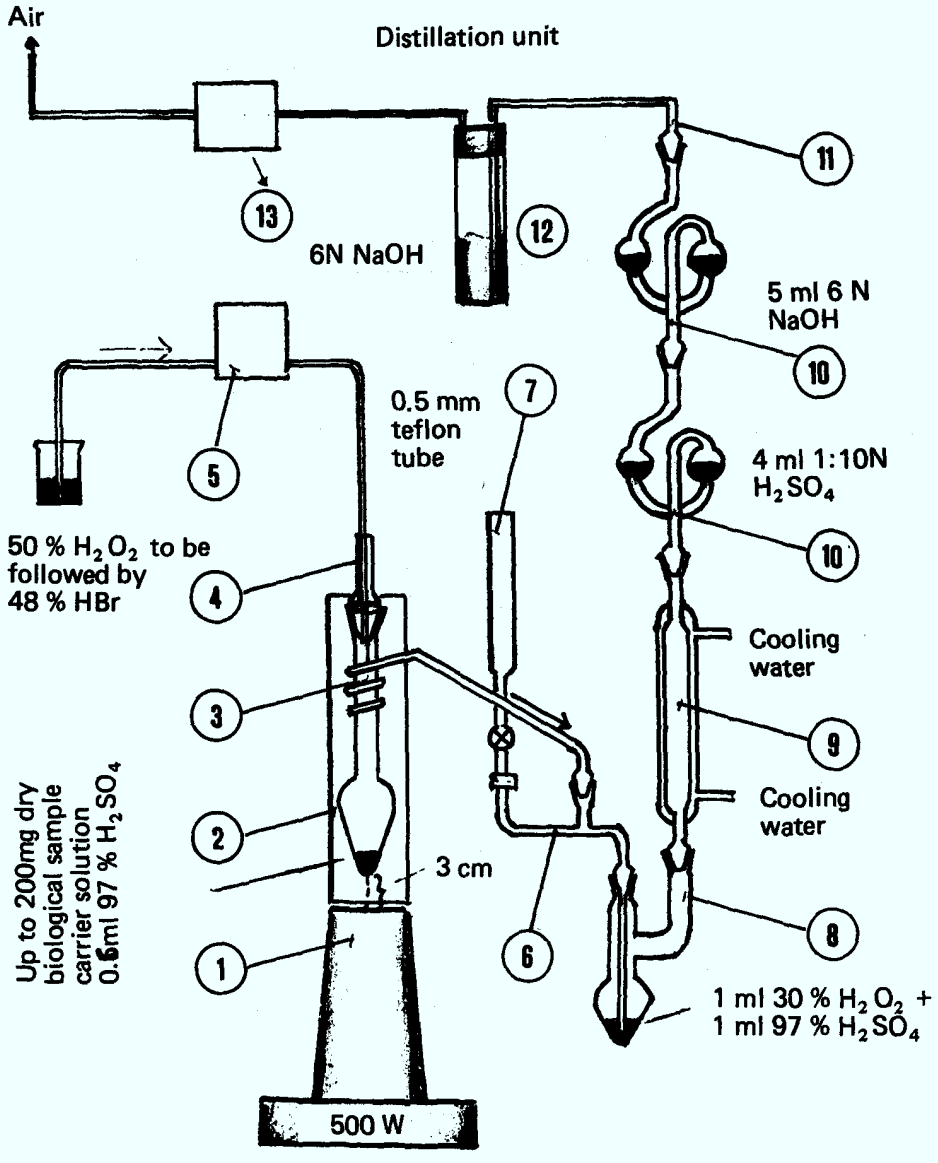
General Scheme of the radiochemical processing

acidic media, pH range, extraction chromatography and specificity of the ion-exchangers including their physical and structural influence on optimum sorption (e.g. mesh size, cross linkage), for a number of elements, has been conveniently combined to group separate a number of elements suitable for gammaspectrometry.

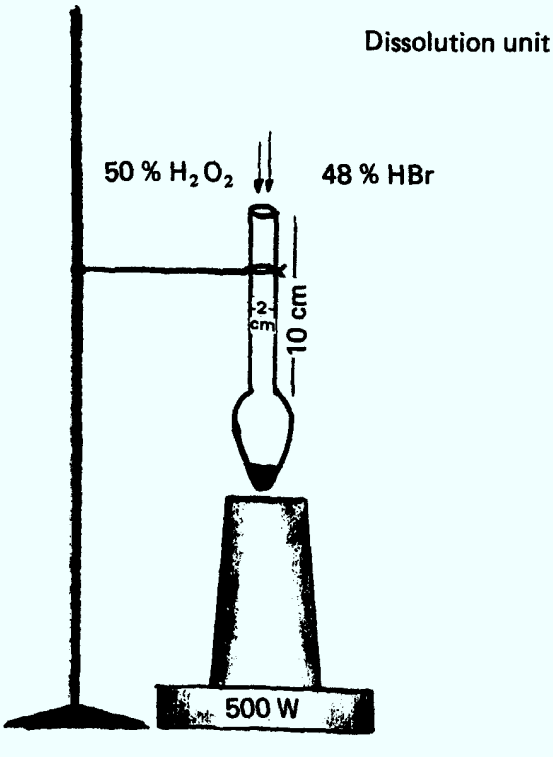
The original separation mode covering a large number of elements is shown in figure 1 . The method involves a matrix destruction stage using concentrated H_2SO_4 and H_2O_2 in a closed system. Following dissolution of the sample, volatile elements like As, Br, Hg, Sb, Se and Sn are distilled away as bromides. The sulphate solution remaining in the sample flask contains the bulk and other non-volatile trace elements of the dissolved matrix. Working steps are as described below.

Sample dissolution: The distillation unit is set-up as shown in the figure 2, filled with various solutions mentioned. Ideally about 200 mg of the dried and powdered irradiated biological tissue is taken in the sample flask. One limiting factor in this context is that of sample size with respect to the total Ca content in the sample, which should not exceed 4 mg, e.g. analysis of bone samples.

50 µl each of carrier solution-1, 2 and 3 are added to the sample, followed by 0.6 ml of 97 % H_2SO_4. Thereafter, the reagent inlet tube is replaced. Air flow through the system is started by operating the suction pump. Cooling water circulation for the condenser is also started. The sample flask is heated strongly for 1 to 2 minutes, keeping a watch on the carbonization. Completion of this step is directly dependant on the nature of the sample. It is advisable to try an inactive run with the type of samples intended to be analysed, to get an idea of the dissolution behaviour. Some samples tend to form lumps and require more heating whereas, fat-rich tissues tend to foam excessively, complicating the dissolution procedure. In any case, these stages should be overcome to obtain a paste like carbonized residue, before starting the H_2O_2 addition. 2.5 ml of 50 % H_2O_2 kept ready to drop into the sample flask is pumped through the reagent inlet line at the rate of 0.8 ml/min. with the help of a micro pump.



1 Electroburner
2 Chimney
3 Sample flask
4 Reagent inlet tube
5 Pump "Ismatec"
6 Connecting tube
7 Air inlet tube
8 Distillate flask
9 Condenser
10 Absorption tubes
11 Suction connection
12 Safety trap
13 Suction pump



If only the non-volatile elements are to be analysed, dissolution of the sample can be carried out in a simple set-up as shown in this figure without any loss due to splattering, etc..
Volume of the sample flask is about 40 ml.

Figure 2:

Heating of the sample flask should be temporarily stopped at the beginning of H_2O_2 addition and restarted when about half a ml of H_2O_2 is added. Usually the matrix destruction is complete by the time the H_2O_2 addition is over. Immediately following H_2O_2, 1.5 ml of 48 % HBr is also pumped through the same reagent line discontinuing heating for a few seconds at the beginning. The somewhat yellow looking residue will be colourless with the addition of a few more drops of 50 % H_2O_2, at the end. The dissolution is now complete, and the sample flask is allowed to cool. Before HBr addition starts, 1 ml of 30 % H_2O_2 is added to the distillate flask through the air inlet tube.

Distillation:The receiver flask is now heated with the electro burner to drive out Br. Incomplete removal of Br will seriously interfere with As and Sb measurement. Heating is continued till the content in the trap containing 1:10 H_2SO_4 is colourless. If necessary, addition of a few more drops of 30 % H_2O_2 will leave the contents of the distillate flask colourless. By experience it has been found that, complete or maximum elimination of Br from the contents of the distillate flask needs about half an hour's heating. Br passes through the H_2SO_4 trap and is absorbed by 6N NaOH. If there is any carry over of Hg vapour passing past the condenser, it is trapped by H_2SO_4. After cooling the system for a few minutes, the H_2SO_4 in the trap is taken back to the distillate flask pouring through the condenser. If necessary, by adding water the solution in the distillate flask is made up to 12 ml.

In a special recovery experiment, it has been proved with NaCl added corresponding to an amount of 20 mg of chloride ions, that volatile chlorides of As, Hg, Sb and Se will not accompany bromine to the NaOH trap. The bromides of these elements are destroyed in the distillate flask because of the excess H_2O_2 present.

Radiochemical separation with SA-7100: The radiochemical machine built as a column-erector set-up is shown in figure 3. The separation scheme incorporated into this machine is shown in the schematic figure 4. The individual working steps for this

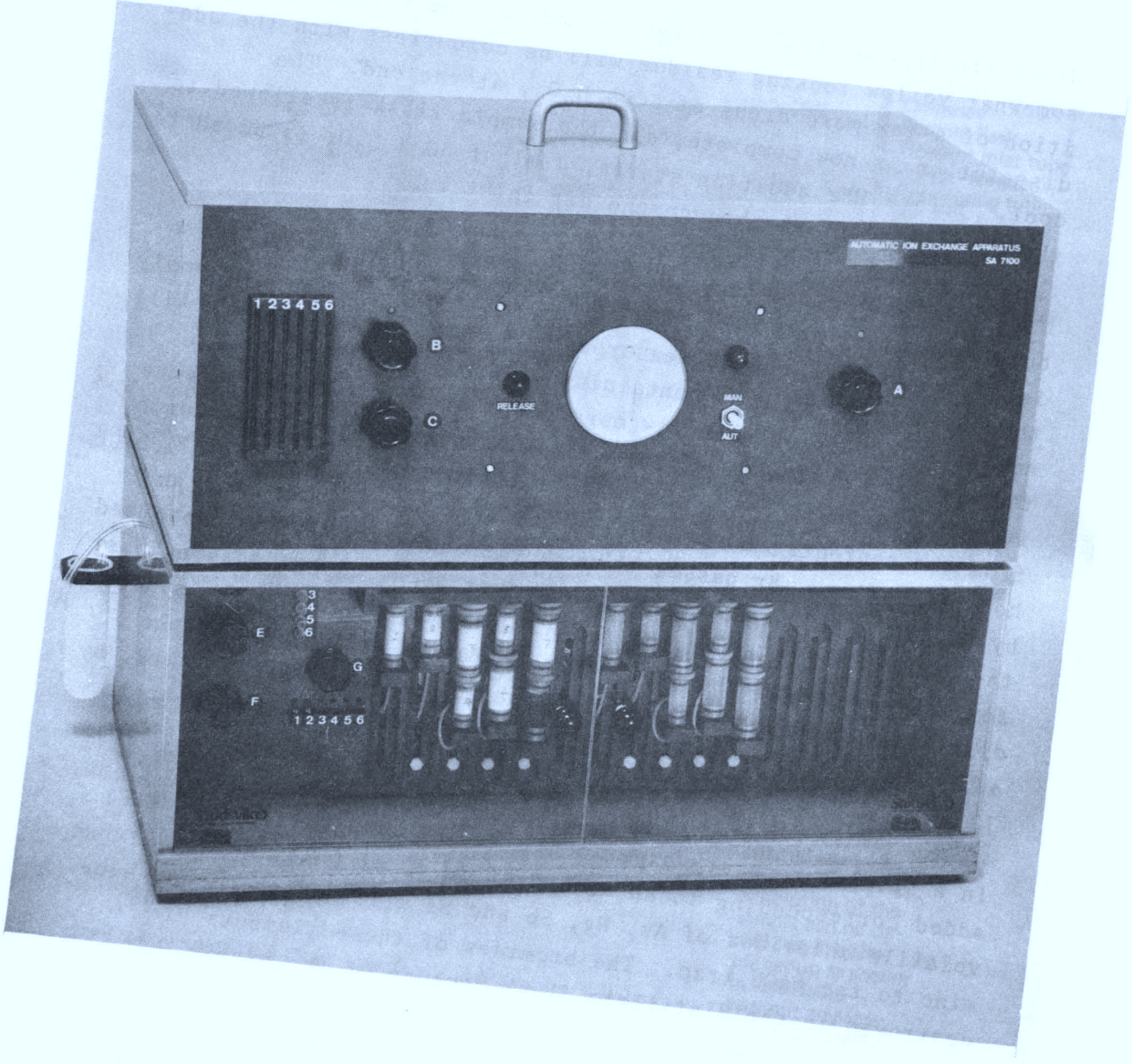


Figure 3: Automatic ion-exchanger apparatus SA-7100

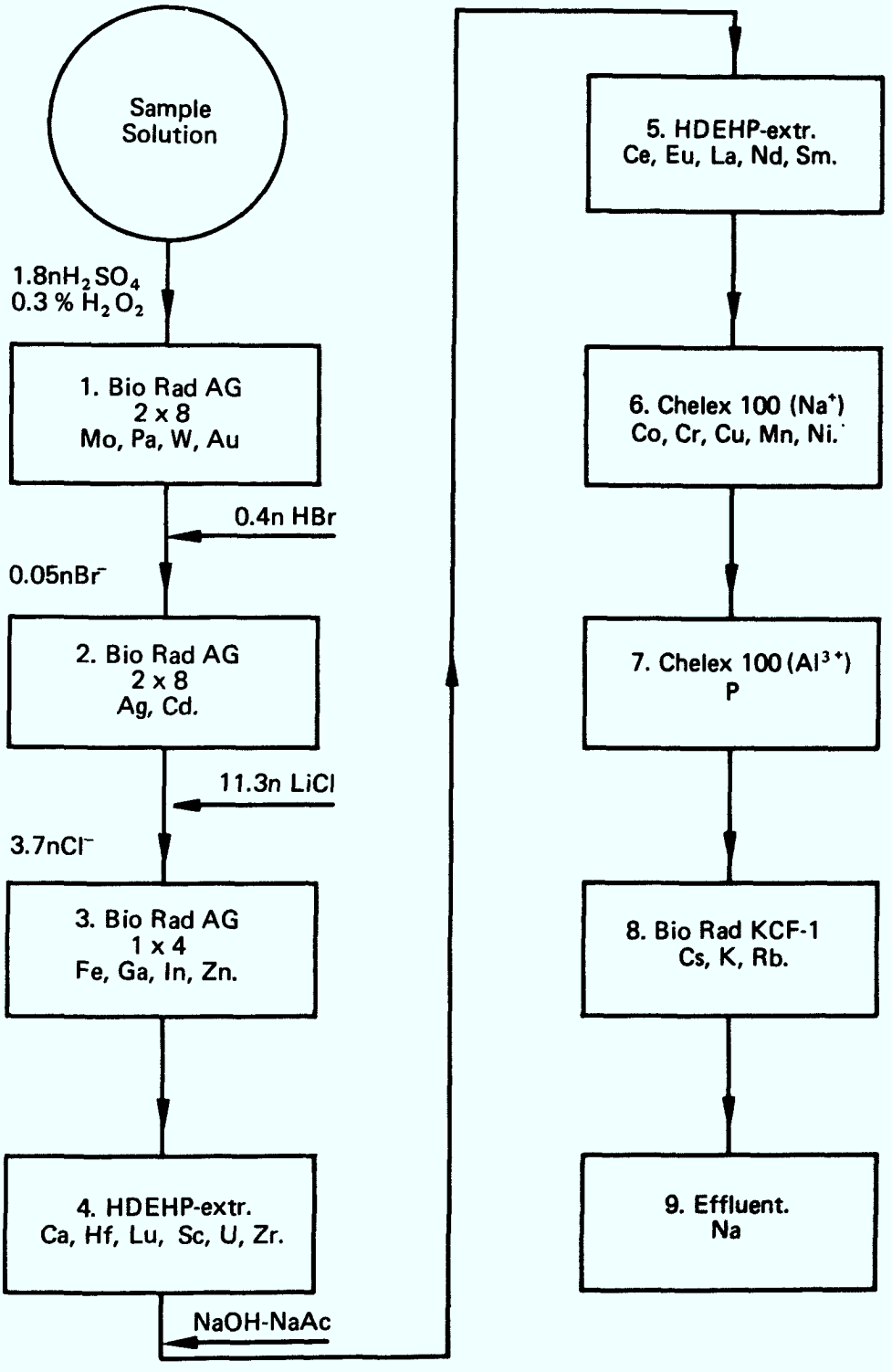


Figure 4:

Radiochemical grouping using SA-7100

machine are described in the manual supplied along with the apparatus. Details of the accuracy and precision studies connected to this apparatus are discussed in (5). This report confines only to the basic chemical and operating features of this set-up. Using a multichannel peristaltic pump which permits independant regulation of the flow at each channel, different process solutions (preparation described under section 3) are pumped through the columns which are connected in series. By choosing the pumping tubes of correct diameter with respect to the solutions of precalculated concentration, desired chemical condition is simultaneously reached for each column, automatically. Wherever there is a new influent to a column, this is mixed with the out-flowing solution of the preceding column in a mixing coil. One of the essential features of this set-up is the uninterrupted and automatic introduction of the sample to the column series without affecting the equilibrium established between the column materials and the influent solutions. The pressure in the system is continuously monitored using a pressure indicator to safe guard against all such hazards resulting from some maloperation. Within the 10 minutes provided for the separation, elaborate washing of the columns with solution of the same influent composition is also included. At the end of this washing process, the machine shuts off automatically. This aspect helps in reducing the radiation hazard to the operating personnel, since it is not necessary to stand near the machine. At present 2 samples can be analysed simultaneously, whereas if only 6 columns are used regularly, provision exists to build up the circuit for another sample. It is of utmost practical importance to clean the apparatus without fail, at the end of each day's work. Figure 5 shows the flow diagram of SA-7100.

It is of practical convenience to keep the machine inside a big plastic tray placed on a turning table. This aspect enables reaching the machine from all the sides and provides an easy mobility to the entire set-up.

Volatile elements: The 12 ml solution remaining in the destillate flask is made up to 20 ml using 12N HCl. It should be noted that after the addition of HCl the separation of As and Se from Hg, Sb and Sn should be completed within 1 to 2 hours, owing to a slow

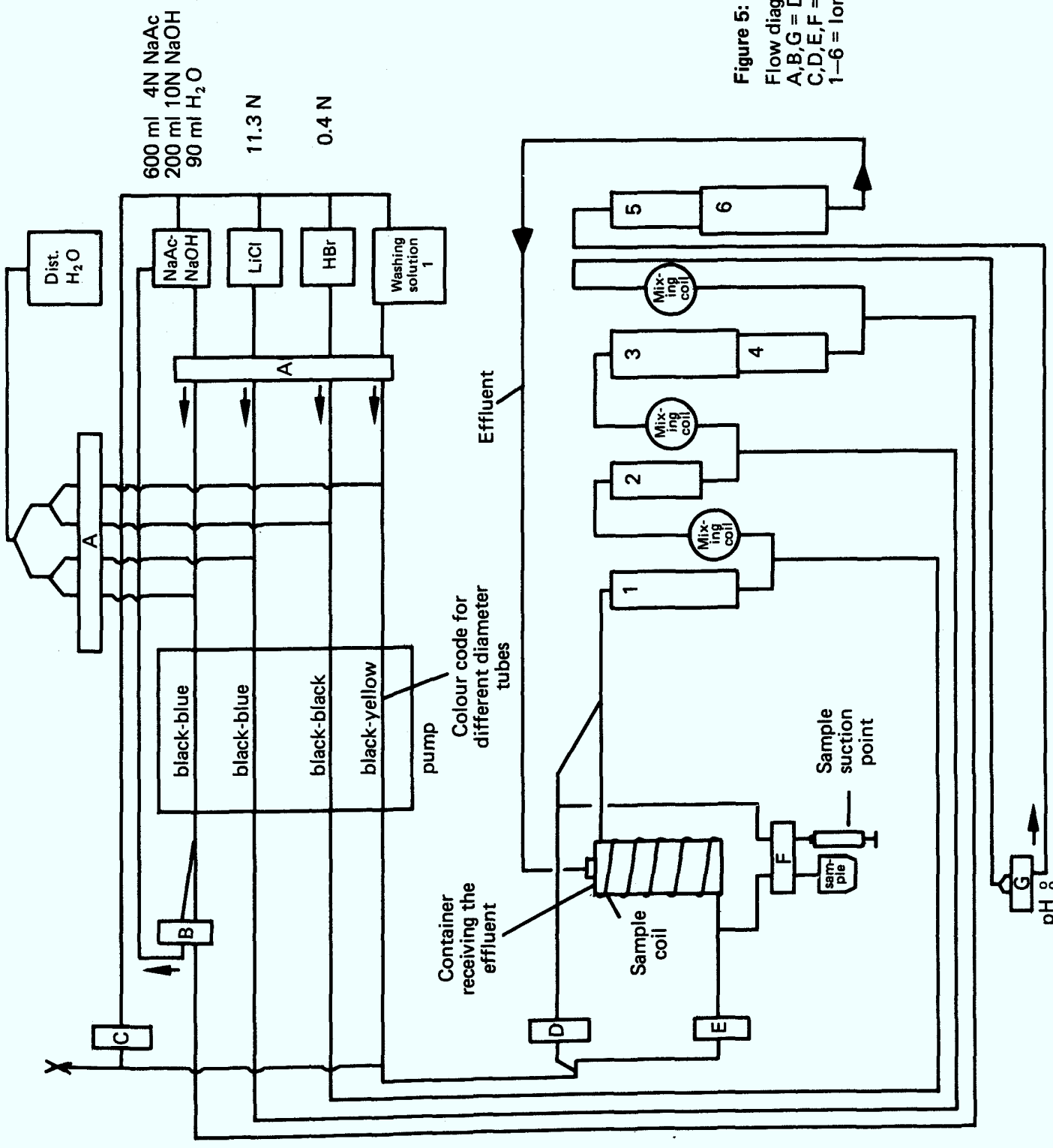


Figure 5:
Flow diagram for SA-7100
A,B,G = Double valves
C,D,E,F = Single valves
1–6 = Ion-exchange columns

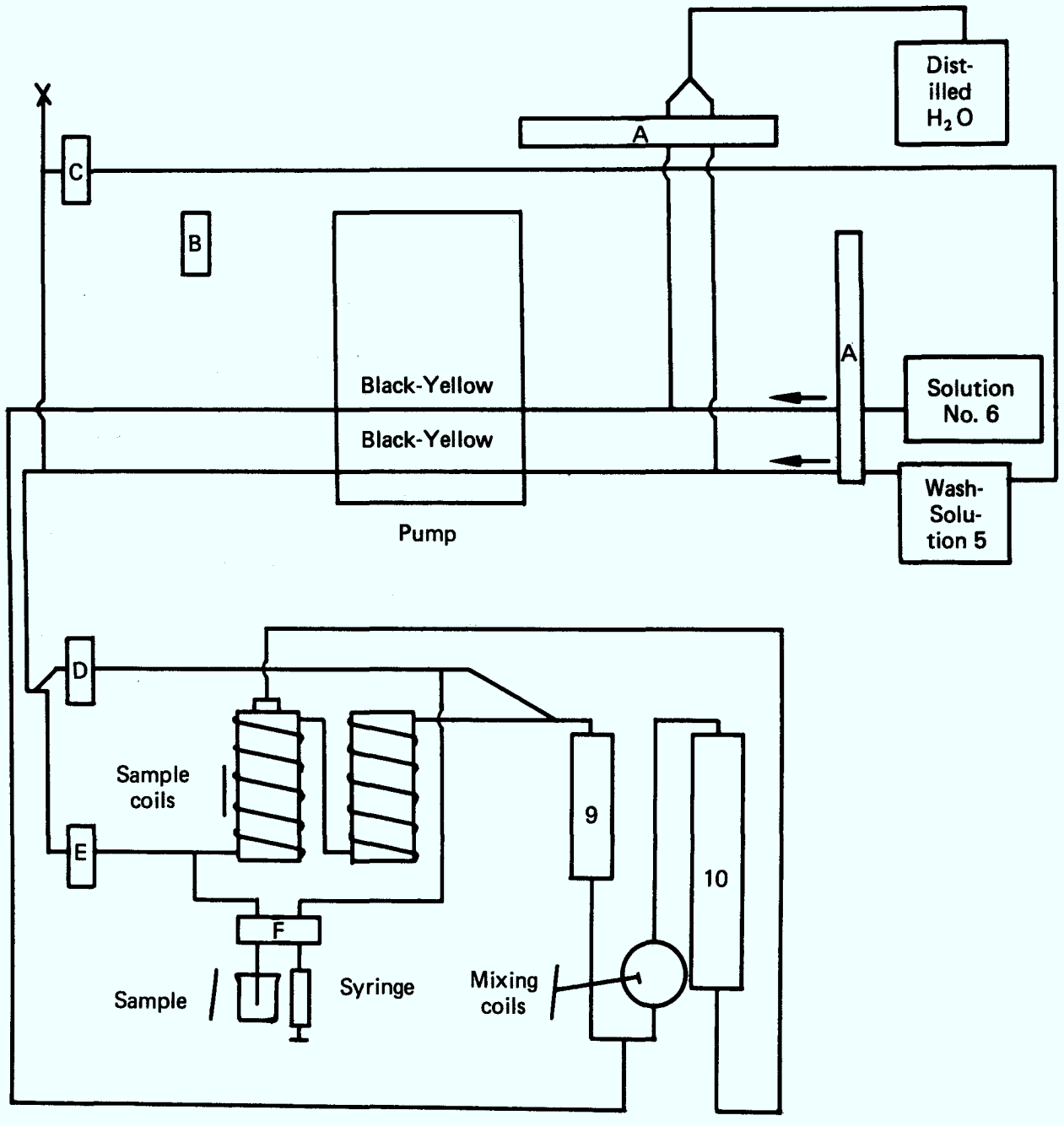


Figure 6:

Flow diagram for the separation of volatile elements
A,B,C: Double valves
C,D,E,F.: Single valves
9,10: Ion-exchange columns

formation of selenium compounds which are not rapidly equilibrating, thus causing an undesired contamination of the Hg, Sb, Sn-group. This separation can be done either by using the SA-7100 or with the help of a simple multichannel pump. The flow diagram of the SA-7100 is shown in figure 6.

If SA-7100 is used, it should be noted that 2 sample coils connected together are necessary in order to accommodate the 20 ml of the sample solution. After the separation, column 9 is washed with a few ml of wash solution 5 and column 10 with a 1:1 mixture of wash solutions 5 and 6.

If a separate pump is used, with the help of a mixing coil the two columns are connected and wash solution 5 and solution 6 are used to equilibrate the columns before pumping the sample solution. The scheme is shown in figure 7. The sample solution can be pumped at 3 ml/min. requiring less than 15 minutes to complete the separation, including pre-equilibrium and washing stages. Using 12 lines of a multichannel pump, 6 samples can be separated simultaneously.

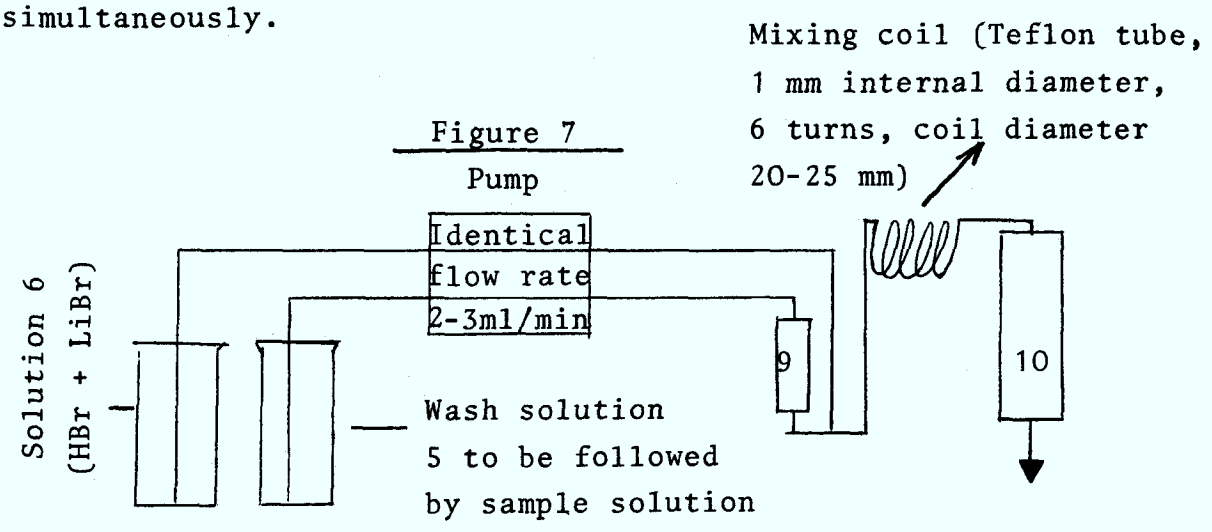


Figure 7

The average recovery values obtained from this procedure (distillation + chemical separation) is between 90 and 100 % for As, Br, Hg and Sn, and 80 % for Se.

Measurement of radioactivity: Generally As, Sn and Hg is measured immediately following separation. Measurement of the long lived ^{75}Se and ^{124}Sb is done after the decay of ^{76}As and the ^{122}Sb

respectively. The quantitative distillation of Br is achieved only, through the use of comparatively large amounts of Br carrier during dissolution and distillation. Further, the use of strong LiBr solution during further subgrouping of the volatile elements through chemical separation is highly effective in increasing the decontamination factor from Br. Yet one practical difficulty in this context is the possible interference of the traces of Br activity, especially while working with samples containing extremely low As content, or very high Br content, like in the case of freeze dried urine or certain plant tissues which are generally rich in bromine. In such cases, a correction due to Br should be applied.

Non-volatile elements

1 ml of 3N NaCl and 8.5 ml of 0.3 % H_2O_2 are added to the 0.5 ml sulphate solution in the sample flask. This solution is sucked into the sample coil of SA-7100, as described in the manual and separation is completed. If columns 7 and 8 are not used, the effluent will then contain Cs, K, Na, P and Rb. The volume of the effluent is measured and 5 ml from this is pipetted into a test tube for radioactivity measurement. Na and K (if K concentration is high enough to overcome the influence of ^{24}Na) radioactivity is measured. Following the decay of ^{24}Na and ^{42}K, Cs and Rb can be quantified. Determination of P by summing up a few bremsstrahlung channels is in principle possible. However, the accuracy of P determination through ^{32}P depends upon the irradiating conditions. If the fast neutron contribution in the thermal neutron column is large, the ^{32}P activity produced by $^{35}Cl(n,\alpha)\ ^{32}P$ and $^{32}S(n,P)\ ^{32}P$ is large because of the high Cl and S concentration of biological tissues. Owing to the big correction factor necessary, it will invariably lead to erraneous results. A typical example is that of Cl, S and P concentrations in blood serum which correspond to 3950, 1220 and 132 mg/l respectively. Where the fast neutron flux is relatively high, unless very rigid corrections are applied, accurate determination of P is questionable.

Removal of ^{24}Na activity: The presence of excess inactive Na^+ ions in the sample and the composite wash solutions, is highly effective in washing away the traces of ^{24}Na activity from the various columns. It is therefore very important that, following separation, the columns are washed with a few ml of corresponding wash solutions on a suction flask. This step also helps in washing away the traces of ^{32}P. Incomplete removal of ^{32}P, because of its intense bremsstrahlung will severely interfere with the peaks at the low energy region of the spectrum, if the desired elements are present in low concentration. It is also advisable to wash the HDEHP columns with a larger wash volume compared to other columns, since the likelihood of ^{32}P retention is higher on these columns.

Measurement of radioactivity:

This is carried out as discussed under geometry factor.

Table 2 summarizes the separation process using SA-7100, ionic species, radioactive nuclides used for identification and the gamma energy times chosen for the peak evaluation.

Complex formed: A detailed discussion on anion exchange selectivity for elements in sulphuric acid media with a strongly basic resin (6), formation of HDEHP compounds (7), complexation aspects using chelating type of resin (8) and finally the halogen complexes formed (9,10) can be found in the cited references.

Table 2

Column or Group	Ionic Species of the elements	Radioactive nuclide measured	Energy in KeV.
1	Au^{3+}	^{198}Au	412
	Mo^{6+}	^{99m}Tc	140
	W^{6+}	^{187}W	686/479
2	Ag^{+}	^{110m}Ag	658
	Cd^{2+}	^{115m}In	336
3	Fe^{3+}	^{59}Fe	1292
	Zn^{2+}	^{65}Zn	1115
4	Ca^{2+}	^{47}Ca	159
	Sc^{3+}	^{46}Sc	889
5	Ce^{3+}	^{141}Ce	145
	La^{3+}	^{140}La	1596/487
6	Co^{2+}	^{60}Co	1172/1333
	Cr^{3+}	^{51}Cr	320
	Cu^{2+}	^{64}Cu	511
	Mn^{2+}	^{56}Mn	846
Effluent	Cs^{+}	^{134}Cs	605/796
	Na^{+}	^{24}Na	1369
	K^{+}	^{42}K	1525
	Rb^{+}	^{86}Rb	1077
9	Hg^{2+}	^{208}Hg	279
	Sb^{5+}	$^{122}Sb/^{124}Sb$	564/603
	Sn^{4+}	^{117m}Sn	158
10	As^{5+}	^{76}As	559
	Se^{6+}	^{75}Se	136/265
6N NaOH	Br^{-}	^{82}Br	776

6. Determination of geometry factor G F

Geometry factor is the ratio obtained by comparing the radioactivity from a known quantity of a particular radionuclide of a certain element in the form of a point source, to the radioactivity measured on the column following radiochemical processing of that particular radionuclide using the same quantity as the standard, and is specific to the detector and the geometry used. These ratios, obtained for all the elements of interest include corrections for recovery in the chemical processing as also for the geometric differences in the counting operations. Columns are generally measured top down to obtain maximum sensitivity.

$$GF = \frac{\text{Radioactivity measured using point standard}}{\text{Radioactivity measured on the column}}$$

When GF values are near unity it means the separated activity is quantitative and that the entire radioactivity is adsorbed on the first few layers of the column, subsequently proving the high sorption selectivity for that element, under those conditions. On the other hand, if the ratio is considerably higher than unity, it means that either the yield is low or that, a particular element is distributed along the column. A good example for the distribution of the element along the column is that of Fe. In such cases, by selecting appropriate column dimensions, the breakthrough point is avoided. Whereas, a still higher ratio is generally the combined effect of both lower yield and down-the-column-distribution, as is usually the case with Cr.

The geometry factors are determined using a synthetic mixture of known amounts of the radioactivities of a number of elements processing like a sample and measuring the separated radioactivity on various columns, using SA-7100.

The working steps are as follow :

a) Non-volatile group
b) Non-volatile but appearing in the effluent stream and
c) The volatile group

a) Non-volatile group

100 µl each of Au, Ag, Ca, Cd, Co, Cr, Fe, La, Mo, Sc,,W and Zn from the stock standard solutions are individually pipetted into cleaned quartz tubes, sealed and irradiated with thermal neutrons for one day, e.g. at a flux of $1x10^{13}$n cm^{-2} sec^{-1}. Only in case of Cu, a few mg of $CuCl_2.H_2O$ is directly irradiated, as the chemical processing starts after 3 to 4 days of decay following the end of irradiation. After the above mentioned decay time, the quartz ampoules are opened and the elements are dissolved individually in different media, as mentioned in Table 1, in about 2-3 ml.

After dissolution, it is advisable to measure the radioactivity level of each of the solutions taking small aliquots. This helps to manipulate the dilution of the radioactivity, to be able to obtain a judicious combination of peak heights of the different radionuclides in the spectrum which will be grouped following chemical processing, e.g. experience has shown that the following approximate range of count rates (in 50 µl aliquots), shown in Table 3 is convenient.

Table 3

Element	Isotope	Half-Life	Counts/min.	Energy KeV
Ag	^{110m}Ag	250 d	1000	658
Au	^{198}Au	2.70 d	1000	412
Ca	^{47}Ca	4.54 d	2000	159 (^{47}Sc)
Cd	^{115}Cd	53.5 h	3000	336 (^{115m}In)
Ce	^{141}Ce	32.4 d	2000	145
Co	^{60}Co	5.26 a	1000	1332
Mn^x	^{54}Mn	312.50 d	1000	835
Cr	^{51}Cr	27.80 d	2000	320
Cu	^{64}Cu	12.80 h	10000	511
Fe	^{59}Fe	44.60 d	2000	1292
La	^{140}La	40.3 h	1000	1596
Mo	^{99}Mo	66.2 h	3000	140 (^{99m}Tc)
Sc	^{46}Sc	83.9 d	1000	889
W	^{187}W	24 h	5000	685
Zn	^{65}Zn	243.8 d	3000	1115

^{x} ^{54}Mn from the stock if available can be used

Preparation of the synthetic mixture:

A 150 ml, long beaker is used for this purpose. 6 aliquots each 50 µl for the sample, together into the beaker, and 3 aliquots separately into different test tubes from each of the radioactive stock solutions are pipetted. 3.5 ml of 97 % H_2SO_4 and 300 µl of carrier solution 1 (see page 5) is added to the sample beaker and the beaker is heated till white fumes of SO_3 just start to appear. At this stage 500 µl of 30 % H_2O_2 is added drop by drop, taking the beaker away from heating to avoid violent splashing of the contents. Following H_2O_2 addition the beaker is heated till SO_3 fumes start to appear. Heating should be controlled at the end stage to prevent the loss of H_2SO_4. The sulphate solution is cooled and 6 ml of 3 N NaCl is added. This solution is made up to 60 ml volume using 0.3 % H_2O_2 and stored in a glass flask with stopper. This solution is used for 6 samples.

b) Non-volatile but appearing in the effluent stream

The required quantity of the solutions (see Table 1) for the elements Cs, K, Na, P and Rb is irradiated and stock radioactive solutions are prepared. Suitable aliquots are directly pipetted into 8 test tubes, giving 8 mixed sources of similar strength. 3 of them serve as point standard sources. The remaining 5 are made 5 ml each in volume using water, to represent the effluent stream from SA-7100. Na and K are measured soon after preparation, and Cs and Rb following the decay of ^{24}Na and ^{42}K. P is evaluated through bremsstrahlung. Comparing the point sources with the 5 ml sources, the factor is determined.

c) The volatile group

The distillation unit is set up as shown in figure 2. 6 ml of 97 % H_2SO_4 + 6 ml of 30 % H_2O_2 are taken in the distillate flask. Suitable quantities of the radioactive solutions of the elements As, Br, Hg, Sb, Se and Sn are pipetted 6 times each and added to the H_2SO_4 in the flask. 3 standard point sources are also prepared, pipetting As+Se as one group and Br, Hg, Sb and Sn separately. 18 mg of the Se carrier (carrier solution-3, page 5) and 300 µg of the mixed carrier solution (carrier-solution-2), are added to the distillate flask. Air and water circulation is started to the system and the distillate flask is heated. When the reaction ceases, 1 ml of 48 % HBr is added to the distillate flask carefully through the funnel. The mixture is gently heated again. Br liberated is absorbed by the NaOH solution in one of the traps. 1 ml of 30 % H_2O_2 is added drop by drop, to drive Br from the distillate flask. Then the flask is cooled, and the 1:10 H_2SO_4 in one of the traps is poured back to the flask through the condenser and contents of the distillate flask are transfered to a measuring cylinder and diluted to 72 ml volume with distilled water. 48 ml of fuming HCl is added to this solution, finally providing 120 ml of sample solution equal to 6 samples. This solution is further processed as described earlier under separation of volatile elements. The radioactivity from the columns and the point sources is measured and compared.

For bromine, the NaOH solution (5 ml) is directly measured and compared with point source to get the factor.

Radiochemical separation:

6 pieces each of columns 1 to 6 are taken from the desiccator where they are stored and using SA-7100 the separation is carried out. Operating instructions are described in the manual.

Measurement of radioactivity:

The columns are measured top down, in the order shown in Table 4. Following the columns, the point standard sources of the respective elements are measured.

Table 4

	Time of measurement	Column	Remarks
1	Immediately after chemical separation	6	for Cu only
2	Immediately after chemical separation	4	Since ^{47}Sc is measured for Ca, correction of ^{47}Sc is necessary up-to the time of separation if the radioactivity measurement is not done immediately after separation on column-4
3	24-36 hours after the chemical separation	1,2	If ^{187}W activity is low, it should be measured soon after separation
4	According to convenience	3	–
5	3 to 5 days after separation	6	Cr and Co

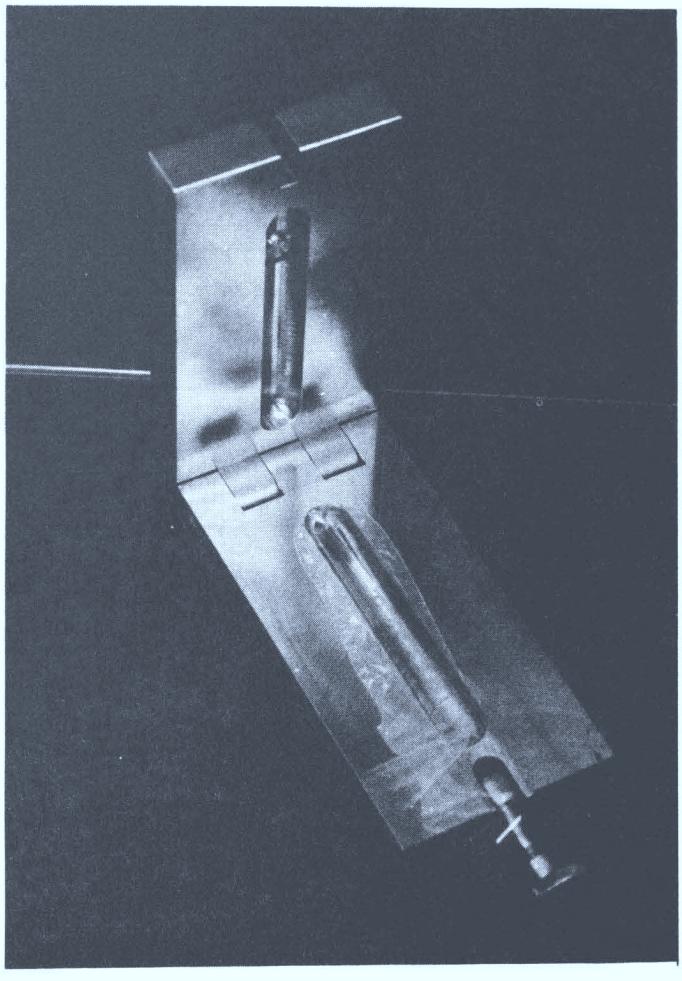
7. Analysis of SRM:

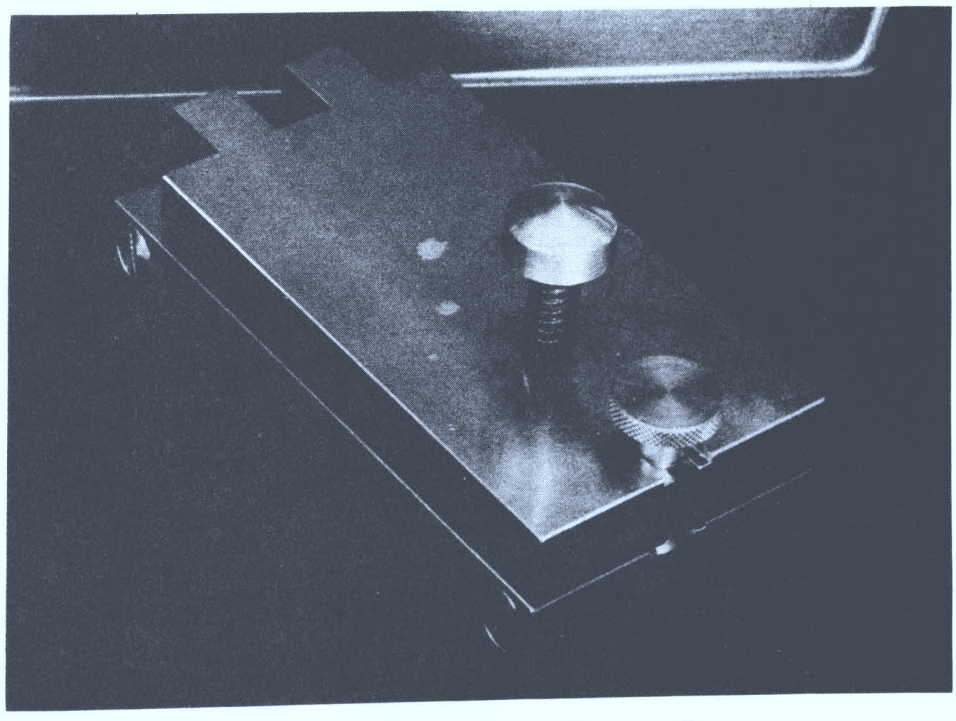
NBS-liver standard and Bowen's kale were analyzed. The values for the non-volatile elements are, using the 90 cm^3 Ge(Li) detector, for which the geometry factors are given. Point standards were irradiated once, along with iron wire and 10 µg of Cu for 24 hours in Merlin reactor, with a neutron flux of $7x10^{13}$ n cm^{-2} sec^{-1}. Thereafter, the samples had only an iron wire and a 10 µg Cu standard for monitoring the flux, and the irradiation conditions were maintained the same. The Fe wire served to monitor the long lived radionuclides in the standard, and Cu to monitor the short lived ones, like ^{42}K, ^{24}Na and ^{64}Cu. The composition of the standards used for this detector is given in Table 5.

The irradiated samples were allowed to decay for 3-4 days. Following this, the surface of the quartz ampoule was cleaned with concentrated HNO_3 and cooled in liquid nitrogen before opening. A specially constructed apparatus made of steel (Kasperek,K and Dresbach,M ., unpublished work) was used to break open the ampoules. The photographs on page 31 show the sample opener.

Values obtained for bovine liver and kale powder: A set of gamma spectra (1 and 2) showing irradiated NBS-liver before chemical separation and another set (3 to 11), following chemical separation using SA-7100, are also enclosed. The positions 1 and 2 mentioned with respect to the geometry, refer to the measurement of the column/sample inside well, and measurement at a fixed position on the surface of the well, respectively. Following values were obtained for the standard reference materials (Table 7).

Animal tissue: Samples from pig muscles were analysed for the elements Zn,Cu and Mn using a simplified version of the separation scheme at a pH of 9 on a 10 x 25 mm chelex column. 16 determinations in all yielded a mean value of Zn=86±3.67, Cu=3.98±0.19 and Mn=0.44±0.024 µg per g on a dry weight basis.





Photographs showing the apparatus used to open the quartz ampoules

Table 5

Mixed-standard combinations and the quantity of the elements in μg.

1	Ag = 1, Cd = 5
2	Au = 0.01, Mo = 1, W = 1
3	Fe = 100, Zn = 5
4	Ca = 500, Sc = 0.50
5	Ce = 1, La = 1
6	Co = 1, Cr = 5, Cu = 10
7	Cs = 1, K = 250, Na = 10, Rb = 25

Calculation of the concentration: Using the geometry factors determined and the ratio of the flux monitor to normalize irradiation conditions the concentration of each of the elements was calculated using the following relation:

$$\text{Concentration in } \mu g/g = \frac{N_2 \times W_1 \times t_1 \times GF \times MF \times 10^3}{N_1 \times W_2 \times t_2 \times \text{Decay correction}}$$

where, N_1 = Number of counts of the point standard
t_1 = measuring time of the point standard
W_1 = weight in μg of the point standard
N_2 = Number of counts of the sample

t_2 = measuring time of the sample
W_2 = weight in mg of the sample
GF = geometry factor of the element determined

$$MF = \frac{\text{Radioactivity from the flux monitor with standard}}{\text{Radioactivity from the flux monitor with sample}}$$

Table 6 shows a set of typical geometry factors obtained for a 90 cm^3 well Ge(Li) detector.

Table 6

Radio-nuclide GF	Energy lines used in this case (Kev)	Radio-nuclide GF	Energy lines used in this case (Kev)
^{110m}Ag-1.18	658	^{59}Fe-1.235^x	1292
^{198}Au-1-20	412	^{42}K -1.25	1525
^{47}Ca-1.25	159 (^{47}Sc)	^{140}La-1.22	487
^{115}Cd-1.00	336 (^{115m}In)	^{99}Mo-1.00	(^{99m}Tc)
^{141}Ce-1.18	145	^{56}Mn-1.10^x	846
^{60}Co-1.18^x	1332	^{24}Na-1.24	1369
^{51}Cr-1.43	320	^{86}Rb-1.20	1077
^{134}Cs-1.20	796	^{46}Sc-1.19	889
^{64}Cu-1.09	511	^{187}W -1.18	686
		^{65}Zn-1.20^x	1115

^{x}In the case of Co, Cr, Cu, Fe, Mn and Zn owing to the smaller diameter of the detector well, the columns could not be measured directly. Hence column material was first homogenised and measured in a test tube inside the well.

Table 7

Element	NBS Liver			Kale powder		
	Present work Mean ± SD		NBS value	Present work Mean ± SD.	n	Bowen's best
Ag	0.071 ± 0.013	n	(0.06)	0.145		-
As				0.11 ± 0.03	8	0.14
Br				24 ± 1	6	26.1
Ca	116 ± 10	4	(123)	38959	1	40850
Cd	0.27 ± 0.035	6	0.27	0.77 ± 0.09	4	0.74
Ce	0.026	2	-	0.21	1	-
Co	0.174 ± 0.022	5	(0.18)	0.059±0.006	4	0.056
Cr	0.112 ± 0.012	7	(?)	0.30 ±0.033	4	0.33
Cs				0.081±0.012	4	0.074
Cu	200 ± 7.6	6	193	5.30 ± 0.31	3	5.0
Fe	260 ± 20	6	270	117.2 ± 12.6	3	120
Hg				0,170±0.008	4	0.167
K	9732 ± 723	4	9700	25814 ± 1532	7	24615
La	0.019	2	-	0.10	1	0.077?
Mn	11.10 ± 0.80	4	10.3	15.50 ± 1	4	14.90
Mo	3.11 ± 0.31	7	(3.2)	2.1 ± 0.13	5	2.16
Na	2385 ± 130	4	(2430)	2559 ± 153	4	2506
Rb	20.1 ± 2.2	5	18.3	50 ± 3	5	52.2
Sb				0.065 ± 0.006	6	0.0589
Se				0.15 ± 0.01	8	0.121?
Sn						
W	0.041± 0.008	3	-	0.073 ± 0.01	4	0.06
Zn	133.2 ± 10.5	5	130	35 ± 2.1	4	33.2

Table 8

8. Approximate number of analytical determination per day using SA-7100
(Estimated for one person, using automatic sample changer for radioactivity measurement and computer for data evaluation)

Elements	Number of samples upto the point of chemical separation per week	REMARKS (Assuming a standard irradiation condition of 1 d irradiation of 100-200 mg of dried biological samples, at 10^{13} n cm^{-2} sec^{-1} followed by 3 d cooling)
Only volatile elements. As, Br,Hg,Sb, Se, and Sn (also Ge)	18 samples irradiated in 3 batches and received for analysis on alternate days	Working procedure: As described under dissolution, distillation of samples and separation of volatile elements. It is practical to finish off data evaluation after 18th sample. Data evaluation and preparation of samples for the next irradiation can be done in one week. Including data evaluation, this would mean 2 weeks, with an average of 12.5 determinations per day.
Only non volatile elements Au,Ag,Ca, Cd, Ce,Co, Cr,Cs,Cu, Fe,K,La, Mo,Na,P,Rb, Sc,W and Zn	8 samples irradiated in one batch received preferably at the beginning of the week	Working procedure: It is practical to arrange for 3 sets of irradiation in advance. Opening the quartz ampoule, weighing the samples and dissolution using a simple flask with a long neck as shown in figure 2, can be finished on the first day. Separation can be finished on the second day including measurement of the radioactivity from the short lived elements. Completion of the radioactivity measurement can be done on 3rd day. The 4th and 5th day can be utilized for the preparation work to analyse the new batch of irradiated samples. 4th and 5th week can be utilized to finish data evaluation, prepare the required process solutions and ion-exchange columns and to arrange the irradiation of new set of samples. The average determinations per day would be 20.
Both volatile and non-volatile elements	6 samples per batch	Working procedure: As described above. Separation and measurement of the distilled group on the day of distillation and the rest of the separation work on the following day, including measurement of ^{64}Cu activity. Total time for 18 samples including data evaluation and preparation of the next batch of samples is about 5 weeks. This would result in an average of 15 determinations per day.

Table 8 (continued)

Some selected elements on single columns. Cu+Zn, Cu+Mn, Cu+Mn+Zn Mo+Cd, etc.	20-30 samples in 2-4 samples per batch	With pre-weighed, freeze dried blood serum as typical example, if the sample is ready for irradiation at the beginning of the day, upto 6-8 samples. (e.g. Cu+Mn see spectrum No.12) can be analysed, providing the results on the same day. These quick procedures are attractive for routine clinical use. 100% retention of Mn on 10x25 mm, Chelex 100, Na^{+} 200-400 mesh size columns was found to be optimum at a pH of 8 to 9.

Note: This is a rough estimate and the number of analytical determinations per person can be considerably improved, if the analysis programme is carefully planned. On the otherhand, it is often experienced that, in a serial analysis determining as many elements as possible, the real limitation is not the number of samples analysed, but the necessary counting time to finish all the radioactivity measurements, before the next batch of samples arrives for counting. This is mainly owing to the generally low concentration of certain biologically important elements, e.g. Cr, Co, Se, Hg, etc. in most of the tissue samples, requiring longer counting time.

Table 9

9. Comparison of instrumental thermal neutron activation analysis with thermal neutron activation analysis using radiochemical separation for two biological samples of clinical interest.

Element	Isotope	(Energy,KeV)	Half-Life	Liver[x]	Serum[x]	Liver	Serum
Ag	110m	658	250 d	0.01	(0.002-0.024)	x0	x0
As	76	559	26.3 h	0.10	0.0002	0	0
Au	198	412	2.7 d	0.00006	0.00004	0	0
Br	82	776	35.4 h	2.60	5.50	x	x
Ca	47 (Sc 47)	159	4.54 d	50	93	0	0
Cd	115(In-115m)	336	53.5 h	220	0.018	x0	0
Ce	141 Ce	145	32.4 d	(0.080)			0
Cl	38	1642	37.3 h	2000	3500	x	x
Co	60	1332	60 a	0.061	(0.0002-0.006)	x	x
Cr	51	320	27.8 d	(0.050-0.27)	(0.012)	0	0
Cs	134	796	2.05 a	0.012	0.003	x0	x0
Cu	64	510	12.8 h	6.70	1.10	0	0
Fe	59	1292	44.6 d	180	(1.09)	x	x
Hg	203	279	46.59 d	0.30	0.003	x	x0
I	128	443	25 m	1.20	0.084	x0	0
K	42	1525	12.36 h	2500	160	x	0
La	140	487	40.27 h	(0.080)	(0.0004)	0	0
Mg	27	844/1014	9.48 m	172	22	0	0
Mn	56	846	2.58 h	1.40	(0.00054-0.005)	x	0
Mo	99(Tc-99m)	140	66.2 h	1.00	(0.006-0.027)	0	0
Na	24	1369	15.0 h	1000	3200	x	x
P	32	Bremsstrahlung	14.2 d	(2020)	142	x	x
Rb	86	1077	18.6 d	3.9	0.71	x	x
Sb	124	564	2.7 d	0.20	<0.006	x	0
Sc	46	889	83.9 d	-	(0.00015)	-	0
Se	75	265	120 d	0.43	0.15	x	x
Sm	153	103	47 h	-	<0.07	-	0
Sn	117-m	158	14.0 d	0.32	0.032	0	0
W	187	686	24 h	-	(0.0004)	-	0
Zn	65	1115	243.8 h	47	(1.10)	x	x

[x]Purely instrumental analysis possible

[0]Through radiochemical separation

[x0]Border line cases. Radiochemistry desirable

[x]These are average concentrations based on µg/g fresh weight. The values are from reference 13, excepting those in parenthesis which are from a recent compilation to be published.

10. Developmental work

Some developmental work was undertaken to improve the recovery values for Cr, examining the possibility to regroup the elements suitable for Ge(Li) detector system and to explore the clinical utility of NAA for elements like Cu, Mn, Zn, etc., for routine use (see page 36).

a. Improved retention of Cr

The need for more information on essential nutrients in human and animal health has increased the analytical interest for Cr in food and biomedical samples. In most of the biological samples, the normal Cr concentration varies from non-detectable levels to a few ng per g. This fact, combined with some of the physico-chemical properties of Cr, often presents analytical challenges for an accurate assay. In this connection, a number of radiochemical procedures are in use, some of which are based on ion-exchange procedures

The chelating type of resins has been finding good applications in chemical separations. These resins consist of a special type of ion-exchangers, in which chelating agents capable of complex-forming reactions are incorporated into the resin matrix. Their analytical properties (8) and special applications are discussed quite comprehensively (11). Some of the properties of this chelating resin have been advantageously used to combine speed and recovery in a conveniently reproducible way, for a set of elements in SA-7100. However, the recovery values for Cr in this set-up are typically in the range of 60 to 70 %, and an improved recovery is desirable, especially when analysing samples containing Cr just above the detection limit. With this in view, an experiment was undertaken to improve the Cr recovery.

Strongly irradiated Cr^{3+} was used to prepare stock 1.8 N sulphate-solution, simulating a biological sample. Known amounts of these sample solutions were then made 3.7 N in chloride concentration using LiCl, followed by adjustment to pH 4, using $NaOH-CH_3COONa$ buffer, which is the prerequisite condition at this stage of the separation in the scheme. Using this solution, the influence of various factors, such as volume of the resin, flow rate and sample dilution, on the retention of Cr using Bio-Rad Chelex 100, 100-200 mesh in Na^+ form (wet mesh 50-100), and Bio-Rad Chelex 100, 200-400 mesh in Na^+ form (wet mesh 100-200) was studied. A peristaltic pump was used to pump the solution. The results obtained as average of two determinations for each run are shown in Tables 10 and 11, as % Cr recovered.

Table 10

Bio-Rad Chelex 100, 100-200 mesh in Na^+ form

Flow rate	Normal solution			With 50% dilution[x]			With 100% dilution		
ml per min.	Col-1	Col-2	Col-3	Col-1	Col-2	Col-3	Col-1	Col-2	Col-3
1	65	90	92	72	92	95	80	95	96
2	57	80	85	60	88	95	70	95	96
4	48	68	72	52	80	85	55	82	92
8	44	56	66	45	64	72	48	71	78

[x]Dilution with water Column length: Col-1=10x25 mm, Col-2=14x25 mm Col-3=14x50 mm.

Table 11

Bio-Rad Chelex-100, 200-400 mesh in Na^+ form.

Flow rate ml per min.	Normal solution			With 50% dilution[x]			With 100% dilution		
	Col-1	Col-2	Col-3	Col-1	Col-2	Col-3	Col-1	Col-2	Col-3
1	80	-	-	85	-	-	88	-	-
2	71	98	100	78	100	100	81	-	-
4	57	91	96	70	95	97	71	-	-
8	43	72	88	50	90	95	48	-	-

[x]Dilution with water

Column length: Col-1=10x25 mm, Col-2=14x25 mm and Col-3=14x45 mm.

The improved retention of Cr seems to be the result of a number of factors, e.g. greater contact time required by Cr^{3+} to enable completion of the exchange, the lowered viscosity of the sample solution, the decreased tendency to form complexes with the acetate ions in the sample solution itself, and above all the better possibility to avoid large volume changes of the resin during separation, as Chelex-100 is very sensitive with respect to swelling characteristics when exposed to solutions of different electrolyte concentrations (12).

Analysis of NBS sample (SRM-1577) using the above retention procedure yielded a mean value of 0.12 $\pm$ 0.0085 ppm Cr values. Other investigators have measured 0.08 to 0.156 ppm for this sample.

Since Cu and Co are adsorbed on the same column in the separation scheme, along with Cr the recovery of these two elements was also tested under these altered conditions. The recovery values for both Cu and Co were always 100 %.

The recovery results indicate that, with 50 % dilution using Bio-Rad Chelex-100,200-400 mesh resin in the normal 14x25 mm column in SA-7100, Cr recovery can be very much improved. Such a change involves a simple 1:1 dilution of the buffer solution with distilled water and use of the same diameter tube as that of wash solution, to pump the diluted buffer. On the other hand, use of 14x45 mm columns to attain maximum recovery should not be discouraging as the column material can be recovered for re-use (12).

B. Regrouping some elements for Ge(Li) detector system

Since the use of Ge(Li) detector is wide spread, the possibility to cut short the number of columns in the series, without forgoing any of the analytical advantages derived by SA-7100, was also briefly examined. This would basically help in saving counting time and the simultaneous possibility to build up the system for more than 2 samples at a time. Preliminary experiments have shown that grouping Ag, Au, Cd, Mo and W on a column of 10 x 25mm, in place of the present column-2 is particularly advantageous, as both Cd and Mo are measured between 24-36 hours following separation. One possible difficulty might arise, if the sample contains an extremely low concentration of W, and the energy resolution of the detector is not high enough. It should be noted that, if the simplified version of the dissolution procedure for the non-volatile elements as shown in figure 2 is used, elimination of ^{82}Br should be carried out, using 48% HBr, added drop wise and heating gently. Cd and Ag peaks may interfere with the 478 and 686 KeV peaks of W, respectively. Another possibility is to combine the elements Ca, Ce, La and Sc on a 10 x 25 mm HDEHP column, in place of the present column-5. Ca(^{47}Sc) and Ce peaks differ by 15 KeV which can be resolved. In the case of very high ^{47}Sc activity, e.g. analysis of Kale and bone samples, use of longer half life of ^{141}Ce still permits the measurement, after sufficient decay of ^{47}Sc. This would mean only 4 columns instead of the present 6 columns. The gamma spectra 10 and 11 show the separation obtained with such a grouping. It is also possible to omit the HDEHP column and complex the elements Ca, Ce, Co, Cr, Cu, La and Sc on the chelex column itself. If so, measurement of Cr has to be done after the complete decay of ^{140}La, as the 327 KeV line

will interfere with the 320 KeV line of ^{51}Cr. Similarly ^{64}Cu should be allowed to decay completely to avoid the compton contribution to the peaks at the lower region of the spectrum. This would require only 3 columns in all in the series, but knowledge of the approximate concentration range of the bulk constituents of the matrix to be analysed is necessary, in such cases. Zn, if present in high concentration, will dominate the spectrum.

Recovery of ion-exchange resins and partition chromatographic column supports.

A general discussion on regenerating column materials for repeated use and certain critical considerations requiring elution recoveries well above 90 % is mentioned in (12). A set of experiments to recover ion exchange resins from the elements Ag, As, Br, Cd, Co, Cr, Cu, Fe, Hg, Mo, Sb, Se, Sn, W and Zn by column and batch experiments were tried using cheap but highly effective regenerating agents. The results of these experiments were used to recover the column materials from SA-7100.

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12. Acknowledgement

The schematic and flow sheet diagrams as well as the distillation sketch included in this report are reproduced from AE-458, AE-389 and the operating manual of SA-7100, with the kind permission of AB-Atomenergi, Sweden.

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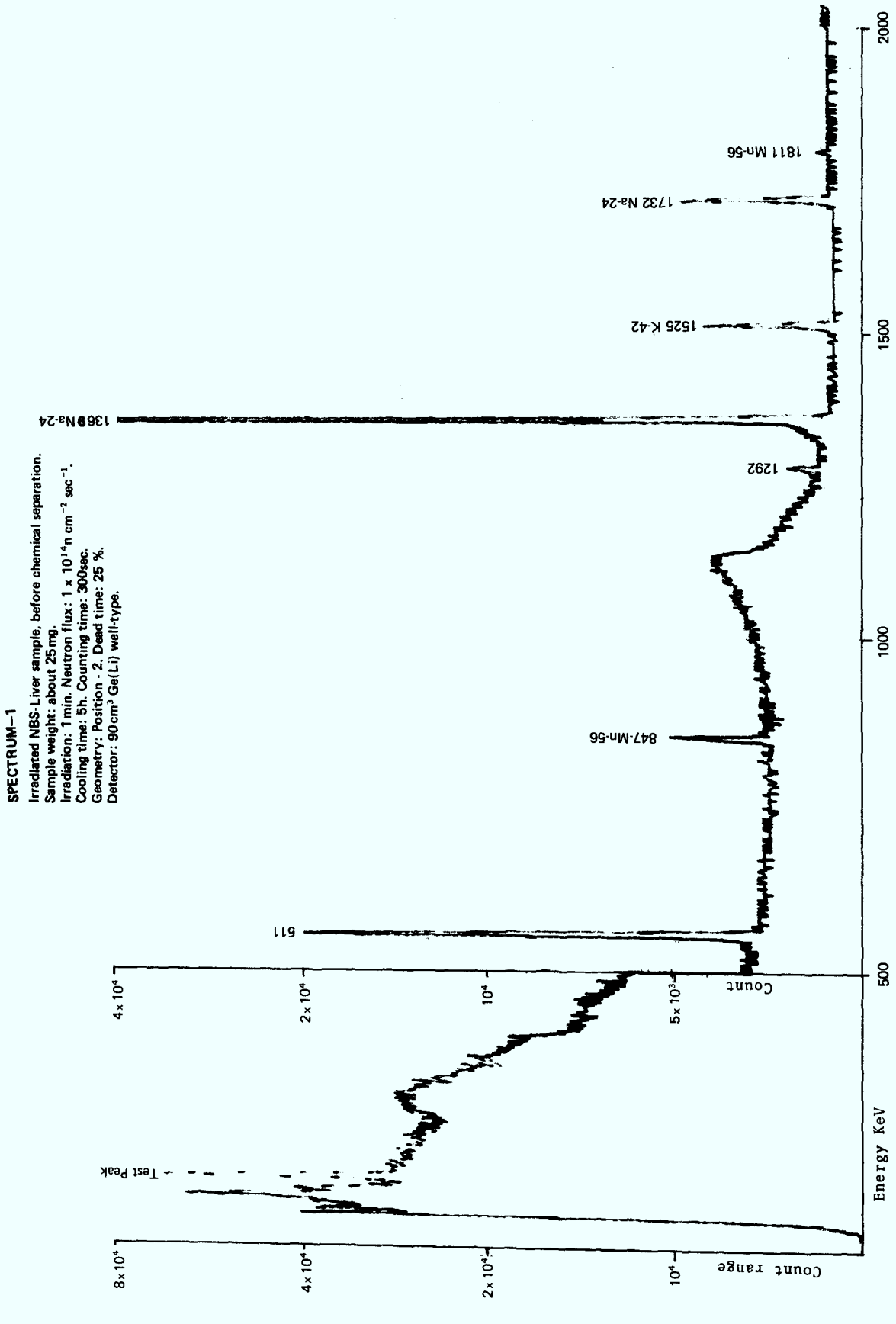
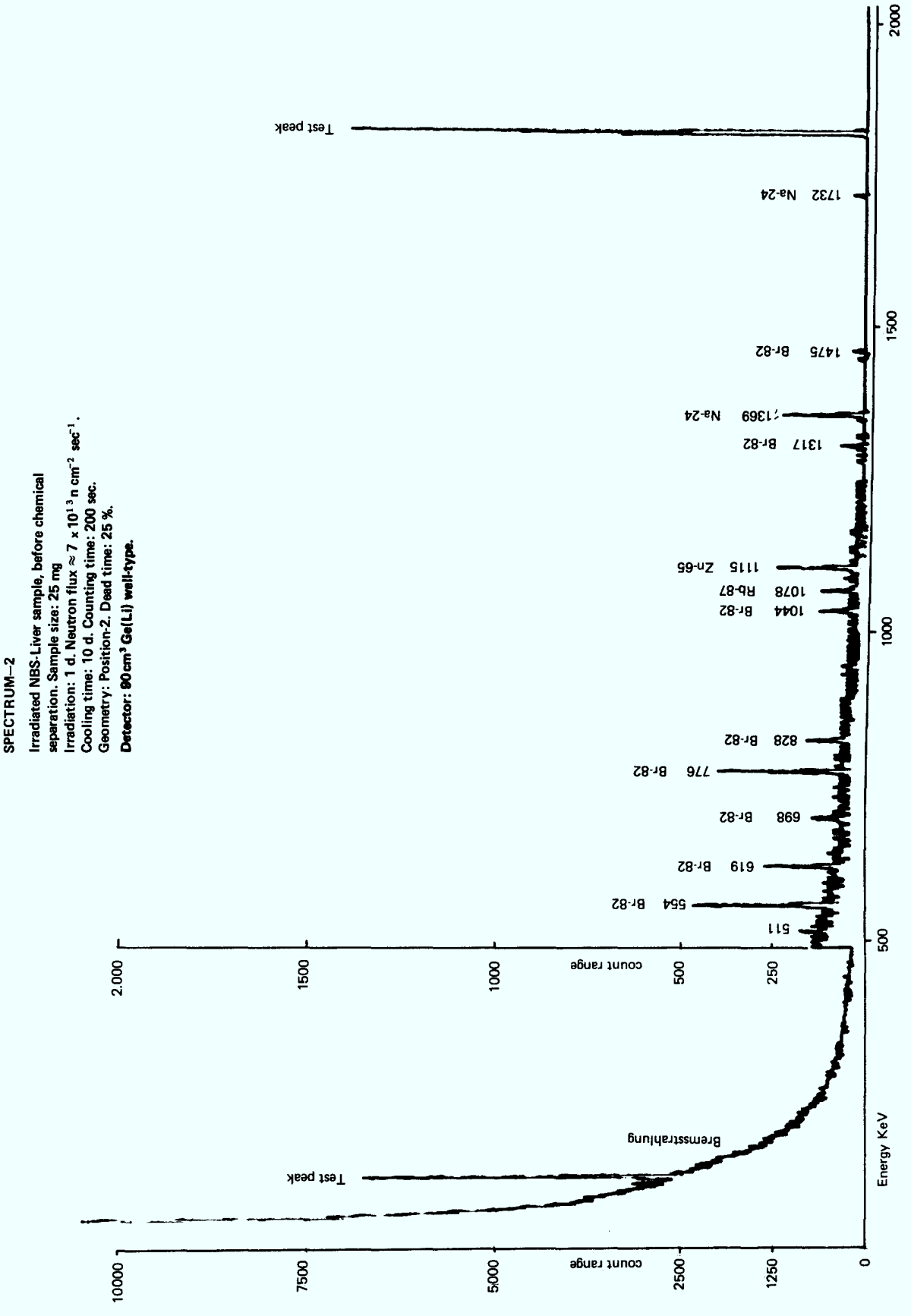
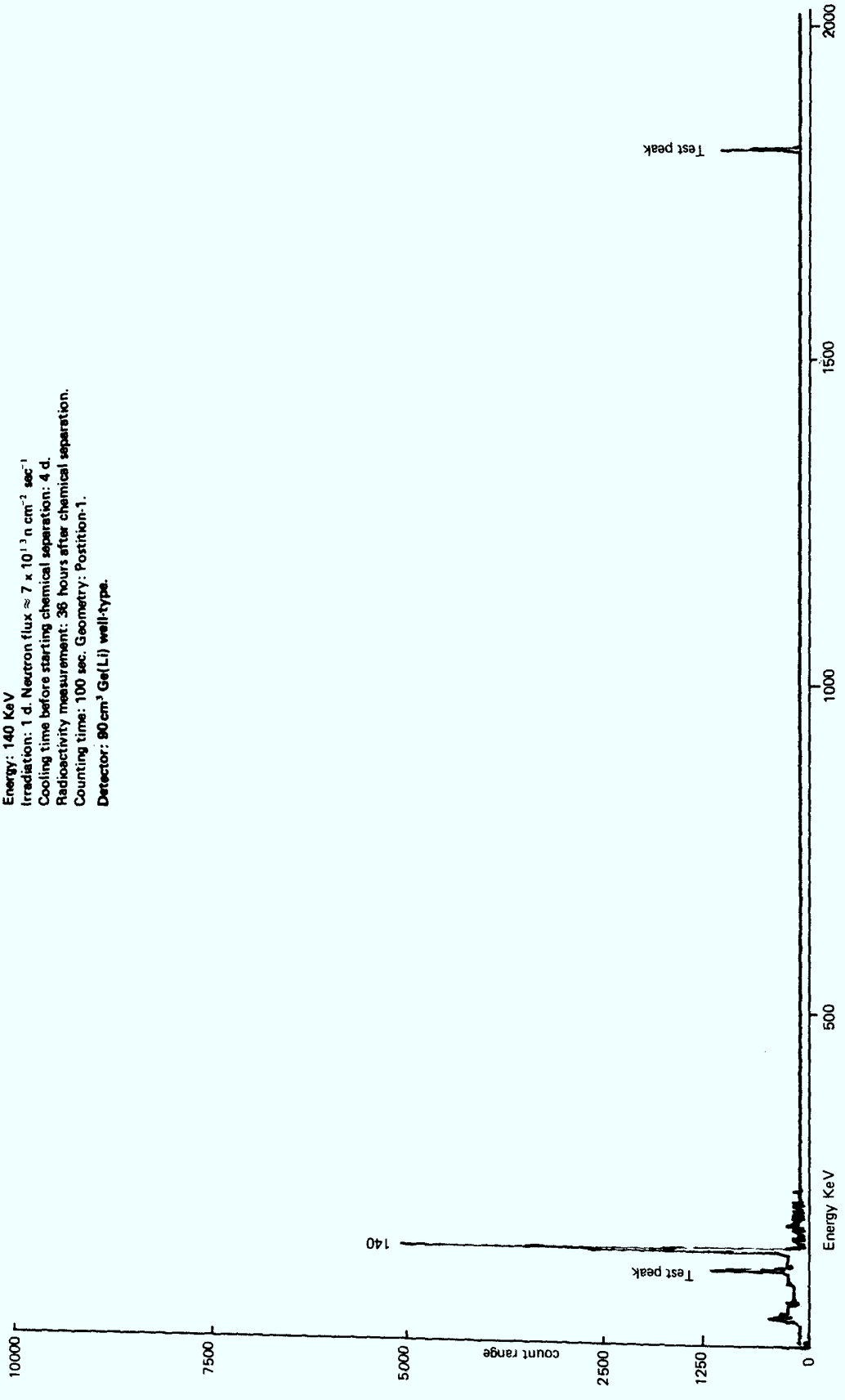

SPECTRUM-1
Irradiated NBS-Liver sample, before chemical separation.
Sample weight: about 25mg.
Irradiation: 1 min. Neutron flux: 1 x 10^14 n cm^-2 sec^-1.
Cooling time: 5h. Counting time: 300sec.
Geometry: Position - 2. Dead time: 25 %.
Detector: 90cm^3 Ge(Li) well-type.
Count range
Count
Energy KeV
Test Peak
511
847-Mn-56
1292
1369 Na-24
1525 K-42
1732 Na-24
1811 Mn-56


SPECTRUM–2
Irradiated NBS-Liver sample, before chemical separation. Sample size: 25 mg
Irradiation: 1 d. Neutron flux ≈ 7 x 10^13 n cm^-2 sec^-1.
Cooling time: 10 d. Counting time: 200 sec.
Geometry: Position-2. Dead time: 25 %.
Detector: 80 cm³ Ge(Li) well-type.
Test peak
Bremsstrahlung
count range
Energy KeV
511
554 Br-82
619 Br-82
698 Br-82
776 Br-82
828 Br-82
1044 Br-82
1078 Rb-87
1115 Zn-65
1317 Br-82
1369 Na-24
1475 Br-82
1732 Na-24
Test peak

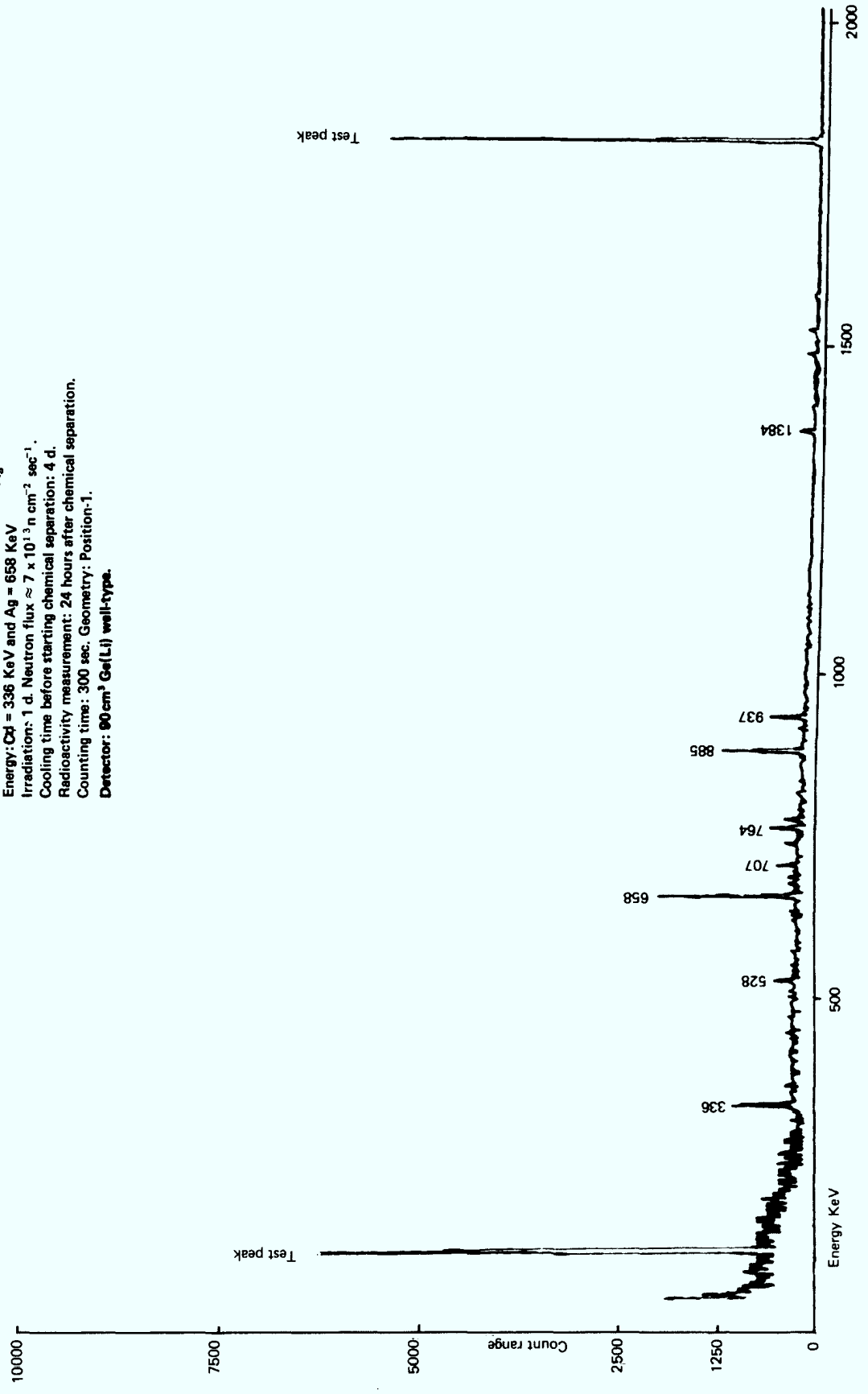
SPECTRUM–3

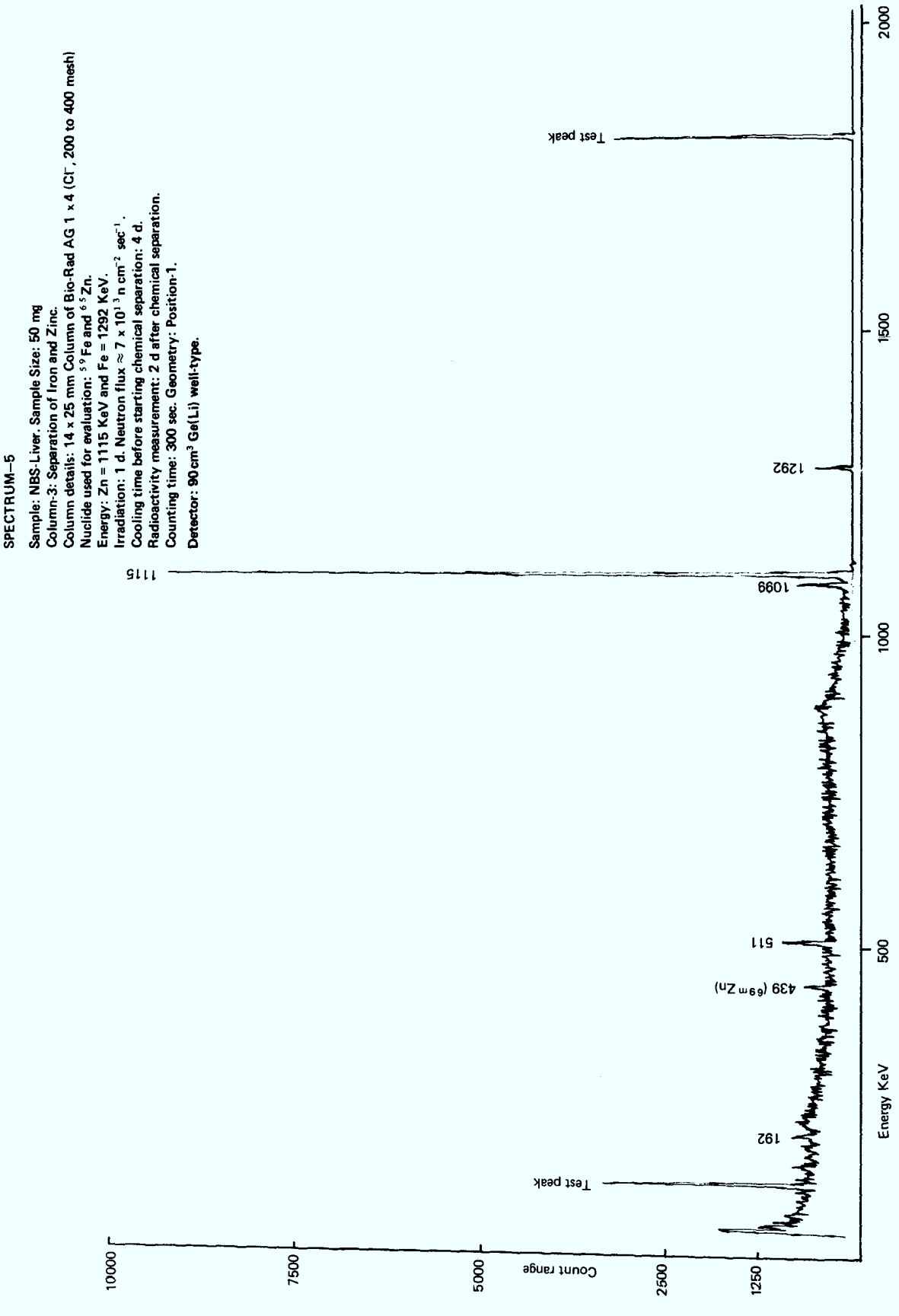
Sample: NBS-Liver. Sample Size: 50 mg
Column-1: Separation of Molybdenum
Column details: 10 x 25 mm Column of Bio-Rad AG 2 x 8 (HSO_4^-, 200 to 400 mesh)
Nuclide used for evaluation: ^{99m}Tc
Energy: 140 KeV
Irradiation: 1 d. Neutron flux $\approx 7 \times 10^{13}$ n cm^{-2} sec^{-1}
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 36 hours after chemical separation.
Counting time: 100 sec. Geometry: Postition-1.
Detector: 80 cm^3 Ge(Li) well-type.

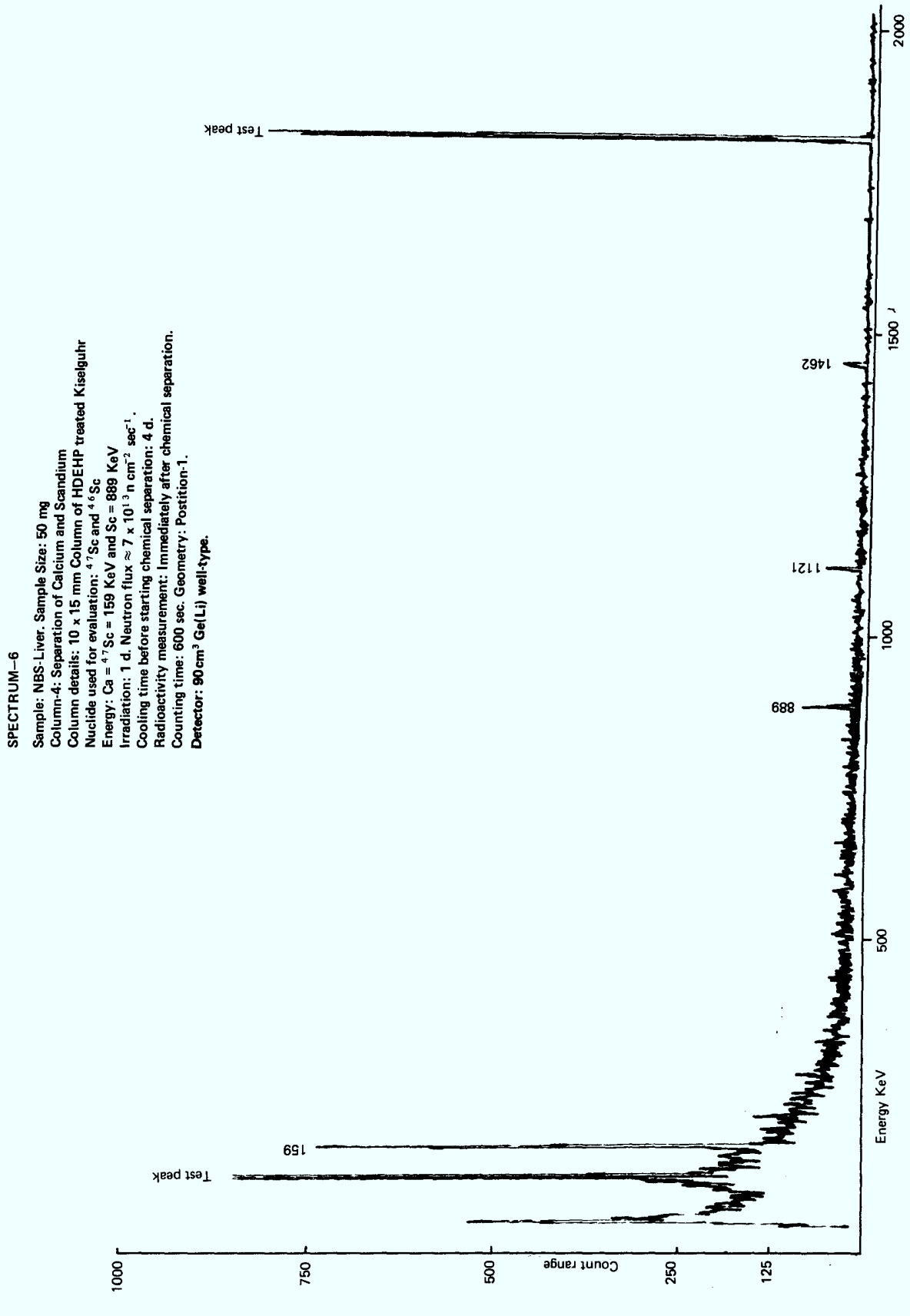


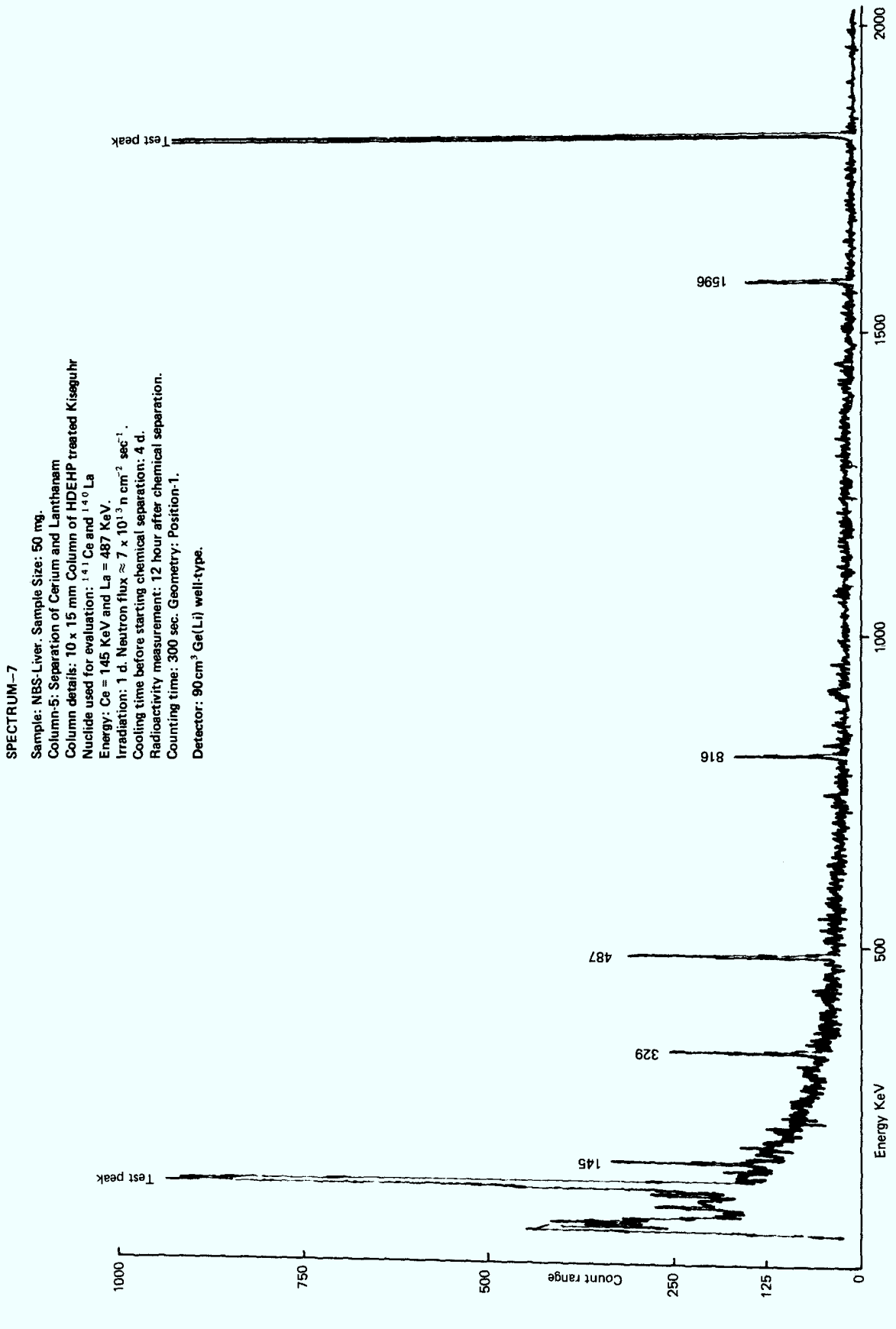
SPECTRUM–4

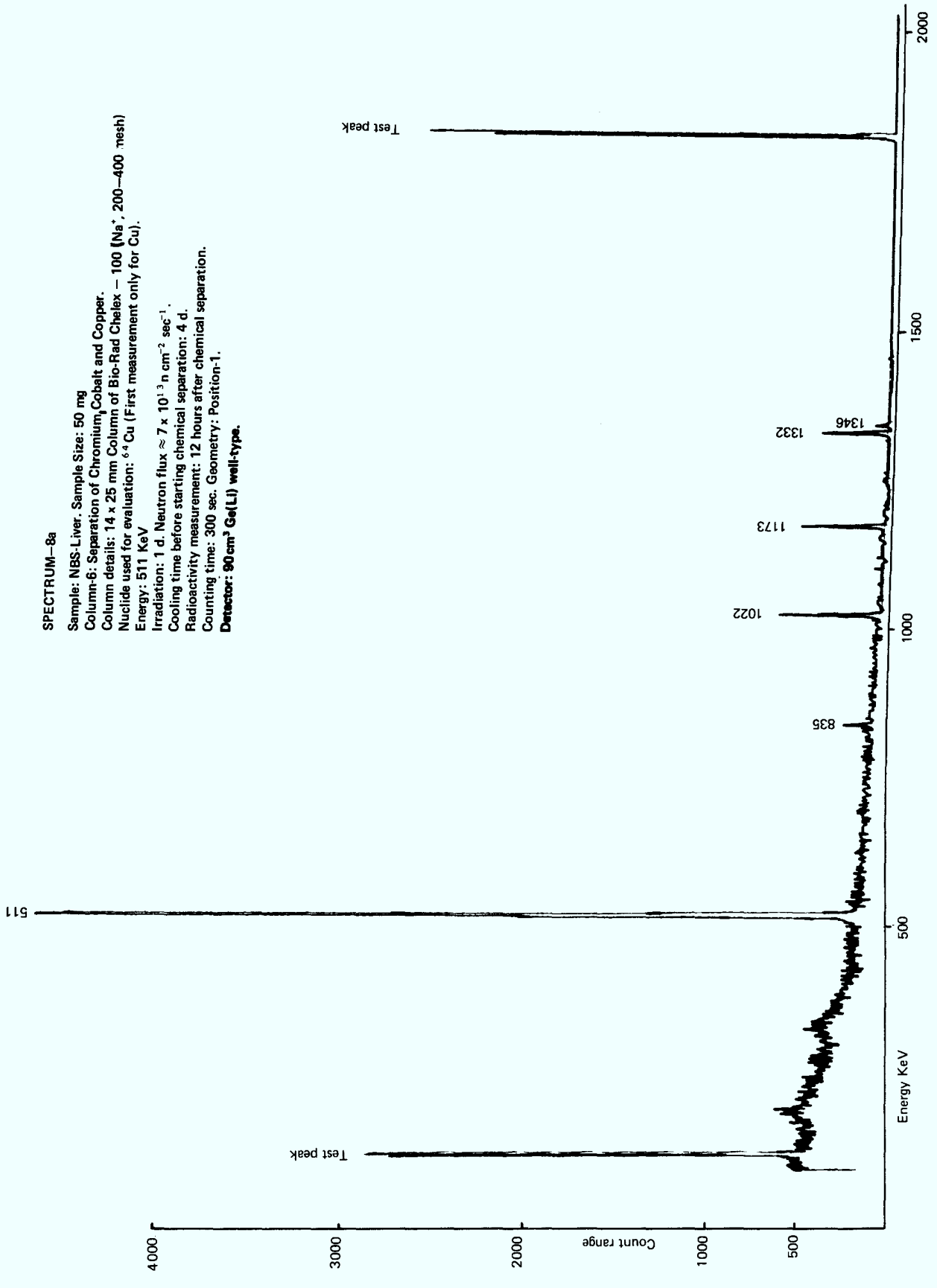
Sample: NBS-Liver. Sample Size: 50 mg
Column-2: Separation of Cadmium and Silver
Column details: 10 x 15 mm Column of Bio-Rad AG 2 x 8 (HSO_4^-, 200 to 400 mesh)
Nuclide used for evaluation: ^{115m}In and ^{110m}Ag
Energy: Cd = 336 KeV and Ag = 658 KeV
Irradiation: 1 d. Neutron flux ≈ 7×10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 24 hours after chemical separation.
Counting time: 300 sec. Geometry: Position-1.
Detector: 80 cm^3 Ge(Li) well-type.

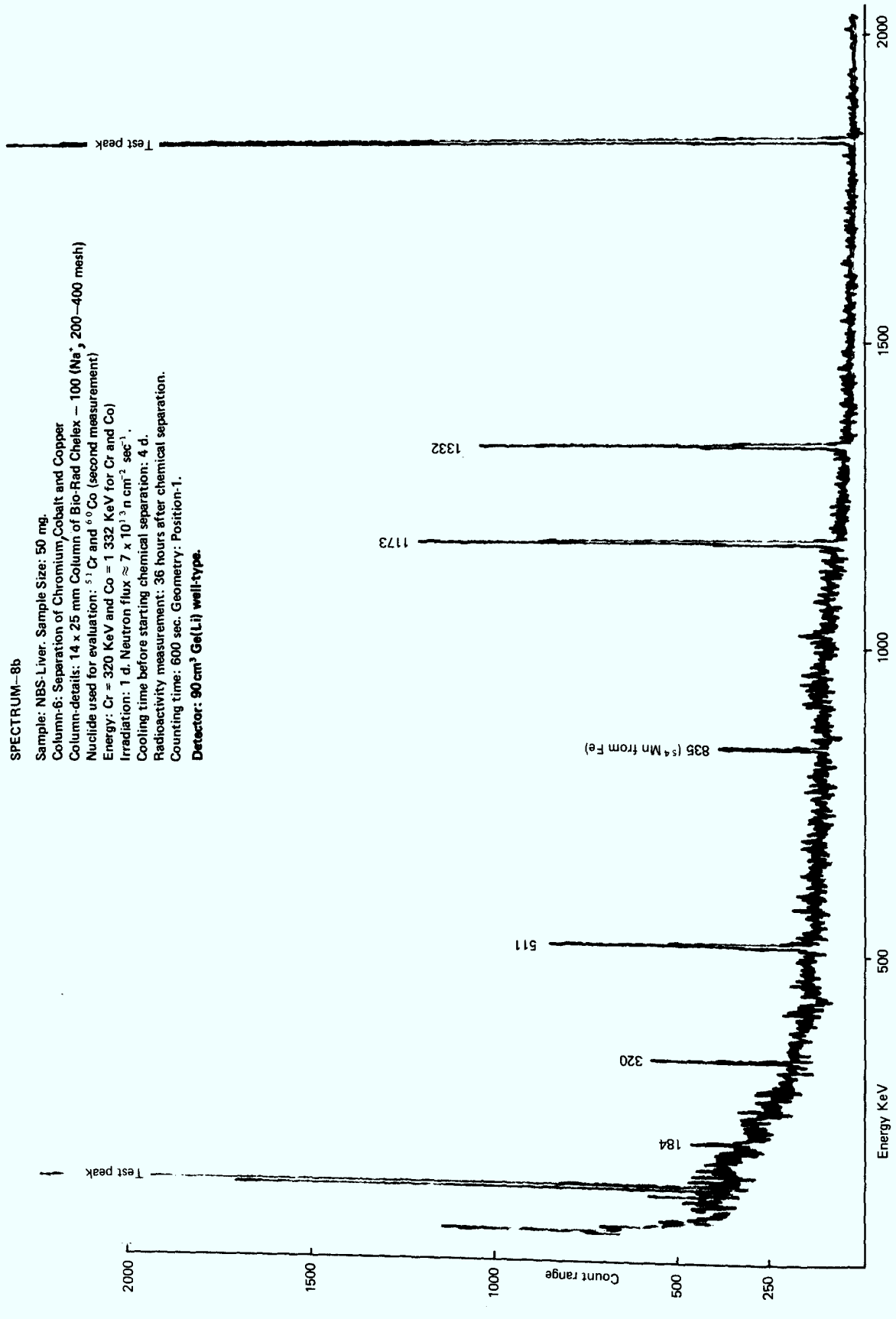


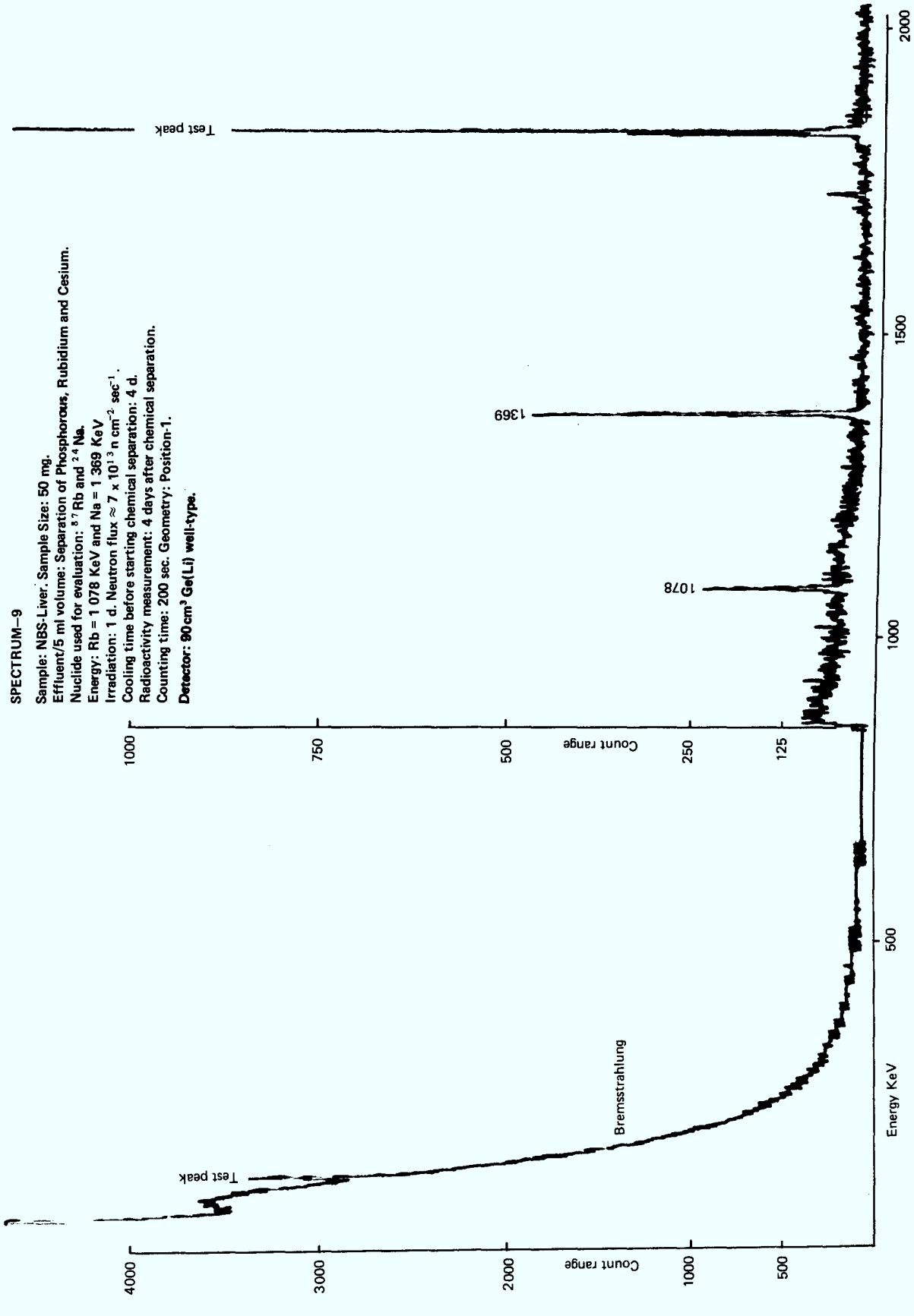

SPECTRUM–5
Sample: NBS-Liver. Sample Size: 50 mg
Column-3: Separation of Iron and Zinc.
Column details: 14 x 25 mm Column of Bio-Rad AG 1 x 4 (CT, 200 to 400 mesh)
Nuclide used for evaluation: ^{59}Fe and ^{65}Zn.
Energy: Zn = 1115 KeV and Fe = 1292 KeV.
Irradiation: 1 d. Neutron flux ≈ 7 x 10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 2 d after chemical separation.
Counting time: 300 sec. Geometry: Position-1.
Detector: 90 cm^3 Ge(Li) well-type.
Count range
10000
7500
5000
2500
1250
Energy, KeV
500
1000
1500
2000
Test peak
192
439 (^{69m}Zn)
511
1099
1115
1292
Test peak


SPECTRUM–6
Sample: NBS-Liver. Sample Size: 50 mg
Column-4: Separation of Calcium and Scandium
Column details: 10 x 15 mm Column of HDEHP treated Kiselguhr
Nuclide used for evaluation: ^{47}Sc and ^{46}Sc
Energy: Ca = ^{47}Sc = 159 KeV and Sc = 889 KeV
Irradiation: 1 d. Neutron flux ≈ 7 x 10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: Immediately after chemical separation.
Counting time: 600 sec. Geometry: Postition-1.
Detector: 90 cm^3 Ge(Li) well-type.
Test peak
159
Test peak
889
1121
1462
1000
750
500
250
125
Count range
500
1000
1500
2000
Energy KeV


SPECTRUM–7
Sample: NBS-Liver. Sample Size: 50 mg.
Column-5: Separation of Cerium and Lanthanam
Column details: 10 x 15 mm Column of HDEHP treated Kieselguhr
Nuclide used for evaluation: ^{141}Ce and ^{140}La
Energy: Ce = 145 KeV and La = 487 KeV.
Irradiation: 1 d. Neutron flux ≈ 7 x 10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 12 hour after chemical separation.
Counting time: 300 sec. Geometry: Position-1.
Detector: 90 cm^3 Ge(Li) well-type.
Test peak
145
329
487
816
1596
Test peak
Count range
1000
750
500
250
125
0
500
1000
1500
2000
Energy KeV

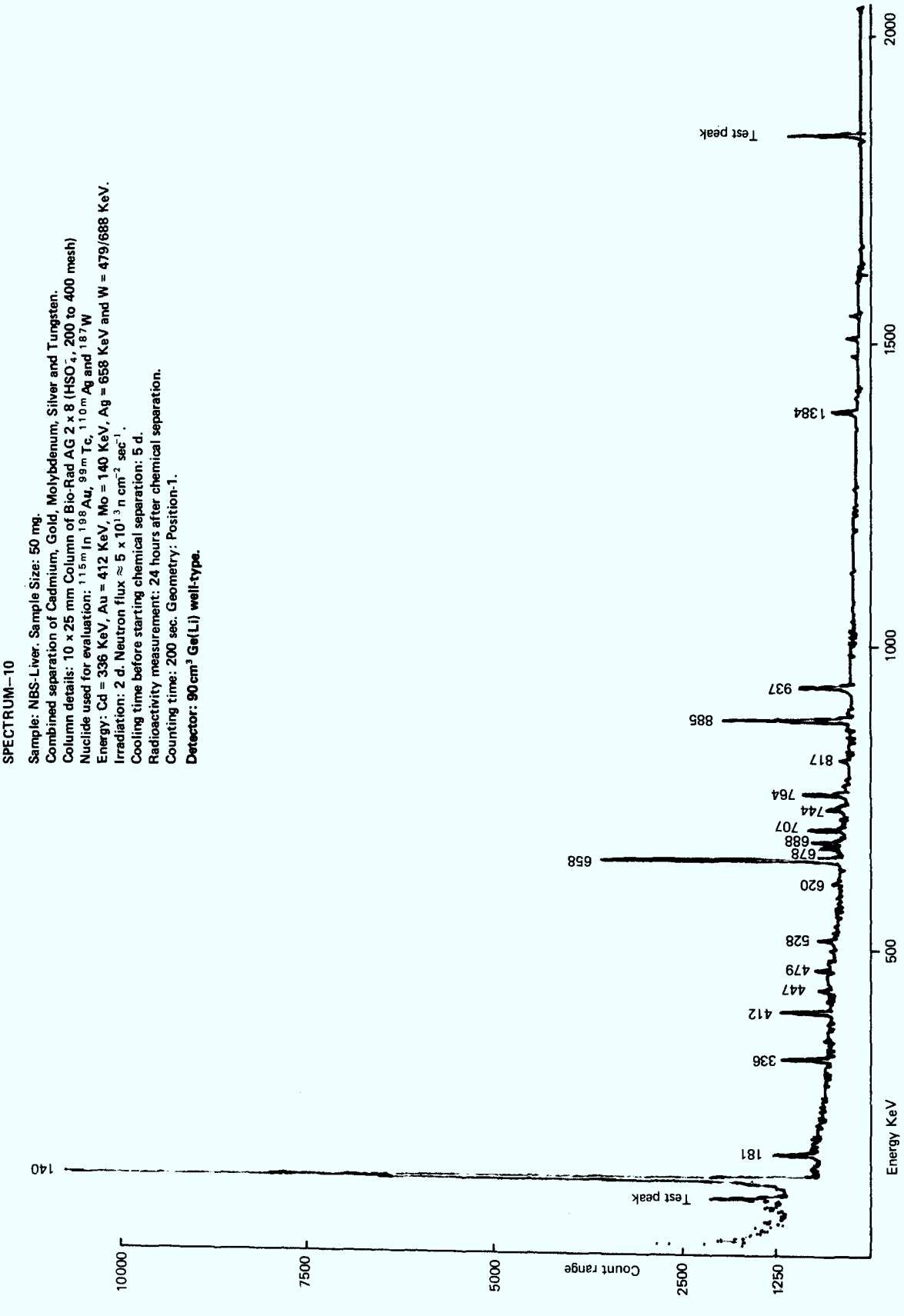

SPECTRUM–8a
Sample: NBS-Liver. Sample Size: 50 mg
Column-6: Separation of Chromium, Cobalt and Copper.
Column details: 14 x 25 mm Column of Bio-Rad Chelex – 100 (Na+, 200–400 mesh)
Nuclide used for evaluation: 64Cu (First measurement only for Cu).
Energy: 511 KeV
Irradiation: 1 d. Neutron flux ≈ 7 x 10-3 n cm-2 sec-1.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 12 hours after chemical separation.
Counting time: 300 sec. Geometry: Position-1.
Detector: 90 cm3 Ge(Li) well-type.
Test peak
511
Test peak
835
1022
1173
1332
1346
Count range
Energy KeV
4000
3000
2000
1000
500
500
1000
1500
2000

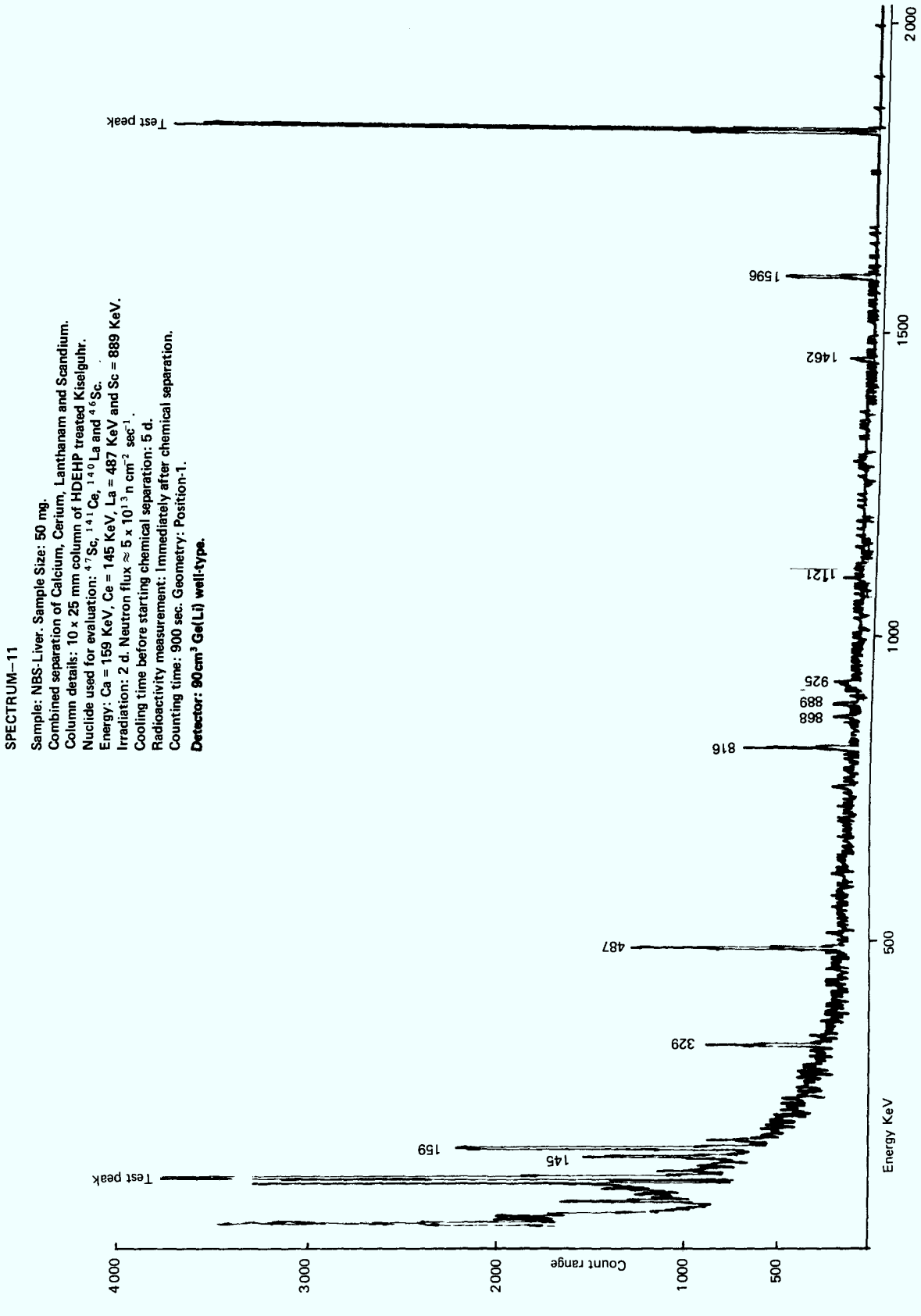

SPECTRUM–8b
Sample: NBS-Liver. Sample Size: 50 mg.
Column-6: Separation of Chromium, Cobalt and Copper
Column-details: 14 x 25 mm Column of Bio-Rad Chelex – 100 (Na+, 200–400 mesh)
Nuclide used for evaluation: 51Cr and 60Co (second measurement)
Energy: Cr = 320 KeV and Co = 1 332 KeV for Cr and Co)
Irradiation: 1 d. Neutron flux ≈ 7 x 10^13 n cm^-2 sec^-1.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 36 hours after chemical separation.
Counting time: 600 sec. Geometry: Position-1.
Detector: 90 cm³ Ge(Li) well-type.
Test peak
184
320
511
835 (54Mn from Fe)
1173
1332
Test peak
Count range
2000
1500
1000
500
250
500
1000
1500
2000
Energy KeV

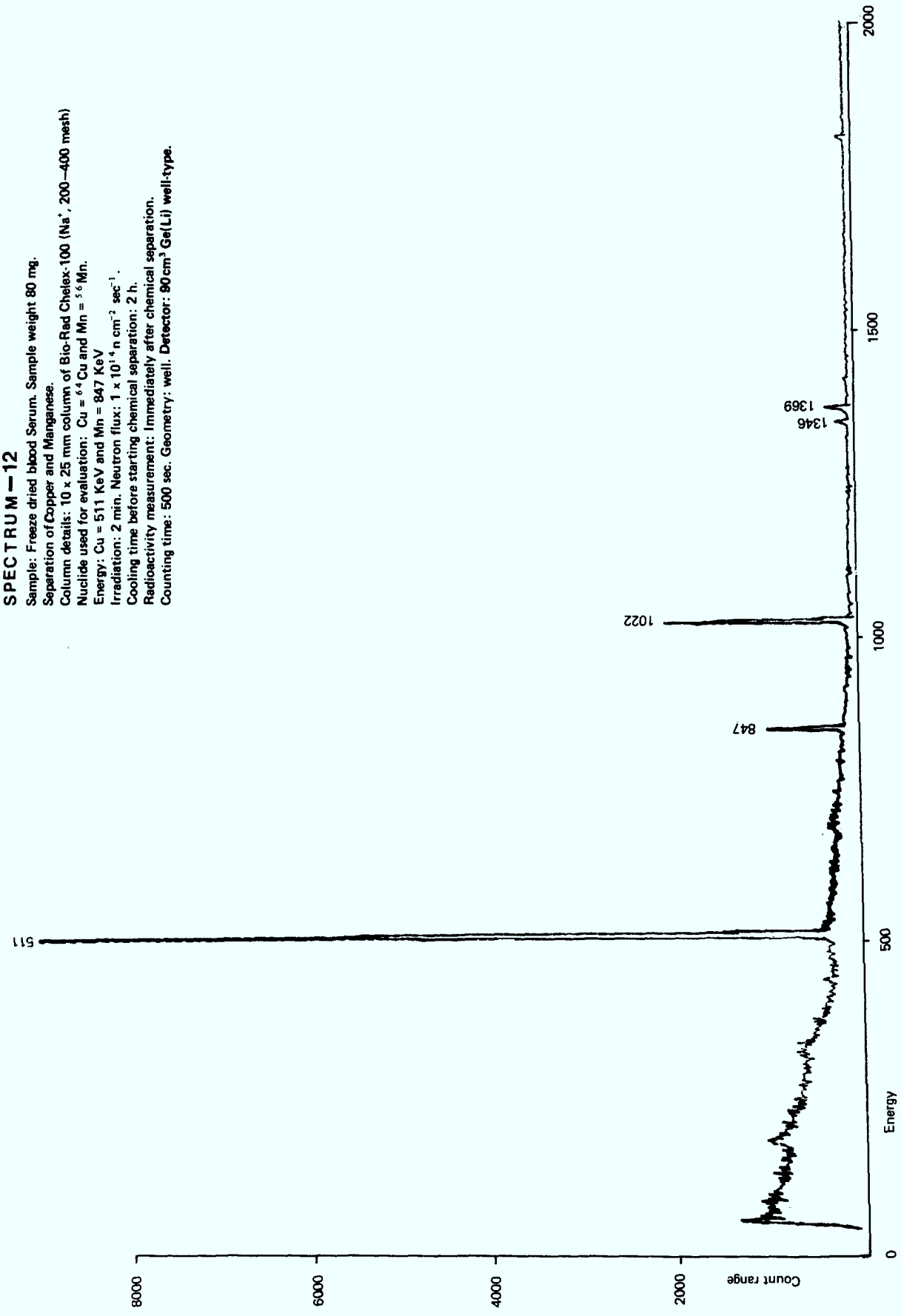

SPECTRUM–9
Sample: NBS-Liver. Sample Size: 50 mg.
Effluent/5 ml volume: Separation of Phosphorous, Rubidium and Cesium.
Nuclide used for evaluation: ^{87}Rb and ^{24}Na.
Energy: Rb = 1 078 KeV and Na = 1 369 KeV
Irradiation: 1 d. Neutron flux ≈ 7 x 10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 4 d.
Radioactivity measurement: 4 days after chemical separation.
Counting time: 200 sec. Geometry: Position-1.
Detector: 90 cm^3 Ge(Li) well-type.
Test peak
1369
1078
Test peak
Bremsstrahlung
Count range
Energy KeV

SPECTRUM–10

Sample: NBS-Liver. Sample Size: 50 mg.
Combined separation of Cadmium, Gold, Molybdenum, Silver and Tungsten.
Column details: 10 x 25 mm Column of Bio-Rad AG 2 x 8 (HSO_4^-, 200 to 400 mesh)
Nuclide used for evaluation: ^{115m}In ^{198}Au, ^{99m}Tc, ^{110m}Ag and ^{187}W
Energy: Cd = 336 KeV, Au = 412 KeV, Mo = 140 KeV, Ag = 658 KeV and W = 479/688 KeV.
Irradiation: 2 d. Neutron flux ≈ 5×10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 5 d.
Radioactivity measurement: 24 hours after chemical separation.
Counting time: 200 sec. Geometry: Position-1.
Detector: 90 cm^3 Ge(Li) well-type.




SPECTRUM–11
Sample: NBS-Liver. Sample Size: 50 mg.
Combined separation of Calcium, Cerium, Lanthanam and Scandium.
Column details: 10 x 25 mm column of HDEHP treated Kiselguhr.
Nuclide used for evaluation: ^{47}Sc, ^{141}Ce, ^{140}La and ^{46}Sc.
Energy: Ca = 159 KeV, Ce = 145 KeV, La = 487 KeV and Sc = 889 KeV.
Irradiation: 2 d. Neutron flux ≈ 5 x 10^{13} n cm^{-2} sec^{-1}.
Cooling time before starting chemical separation: 5 d.
Radioactivity measurement: Immediately after chemical separation.
Counting time: 900 sec. Geometry: Position-1.
Detector: 90cm^3 Ge(Li) well-type.
Test peak
1596
1462
1121
925
889
868
816
487
329
159
145
Test peak
Count range
4000
3000
2000
1000
500
Energy KeV
500
1000
1500
2000


SPECTRUM—12
Sample: Freeze dried blood Serum. Sample weight 80 mg.
Separation of Copper and Manganese.
Column details: 10 x 25 mm column of Bio-Rad Chelex-100 (Na+, 200—400 mesh)
Nuclide used for evaluation: Cu = 64Cu and Mn = 56Mn.
Energy: Cu = 511 KeV and Mn = 847 KeV
Irradiation: 2 min. Neutron flux: 1 x 10^14 n cm^-2 sec^-1.
Cooling time before starting chemical separation: 2 h.
Radioactivity measurement: Immediately after chemical separation.
Counting time: 500 sec. Geometry: well. Detector: 80cm3 Ge(Li) well-type.
Count range
8000
6000
4000
2000
0
Energy
500
1000
1500
2000
511
847
1022
1346
1369