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AN ION-EXCHANGE GROUP-SEPARATION SCHEME FOR RAPID ANALYSIS OF THE COMPONENTS OF NEUTRON-ACTIVATED BIOLOGICAL TISSUES

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A simple ion-exchange separation scheme has been developed for the separation of over 50 constituent chemical elements in biological tissues into 12 to 15 groups suitable for quantitative gamma-ray spectrometry. The scheme incorporates several important improvements and modifications of earlier radiochemical ion-exchange separation procedures, and allows rapid simultaneous quantitative analyses of a large number of constituent components in tissues. The procedures can easily be adapted for use with a variety of other materials and mixtures. The components of the various fractions are listed and their gamma-spectrometric analysis is discussed. The separation is comparatively quick, and yields clean and easily-identifiable components.

Introduction

The complexities of gamma-ray spectra from multielement analysis of biological samples often prevent the simultaneous determination of a large number of elements by instrumental methods following neutron activation. The high activities produced by some abundant high activation cross-section isotopes frequently distort or mask the lower activities from isotopes of low cross-sections and/or long decay half-lives. A radiochemical separation procedure must be employed to separate the individual gamma-activities into single components, or into several groups with limited numbers of activities, to effect a reliable quantitative analysis, or at least to confirm the results of a high-resolution instrumental gamma-spectrometric procedure.

The separation procedure must be rapid, in order not to lose the data for appreciable numbers of short half-life components, reasonably simple to minimize procedural errors, and, of course, quantitative. Above all, it must succeed in the separation of the components having overlapping gamma-energies or nearly identical decay half-lives, into non-interfering groups.

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Several schemes have been proposed for multielement separation in biological samples, some of them very complex.[1-4] A simpler, modified version of a combination of group-separation schemes by Wong[5] and Morrison[6] has been developed in this work for use with a large variety of biological tissues of animal or human origin.[7]

Experimental

Apparatus and reagents

The ion-exchange columns were prepared from quartz tubing, 0.8 cm in inside diameter, in 8 cm lengths. A small wad of glass wool was introduced at the bottom of the column to hold the ion-exchange resin, and the tubes were rinsed with silicone oil [$Si(CH_3)_3Cl$ in cyclohexane] to minimize adsorption losses. Similarly-treated glass vials were used for the collection of the eluents. These materials were also satisfactory when low concentrations of HF were used in the elution process, with the exception that the glass wool plugs had to be periodically exchanged. When HF concentrations above 1–2N are to be used for further refinement of any of the separation steps, polyethylene tubing with a plug of teflon, lucite or cellulose shavings must be substituted for the glass.

It was not necessary to use superpure analytical grade chemicals in the procedure, as is necessary in pre-irradiation processing of samples, because any contaminants introduced are inactive and do not interfere with the measurement of the activated components.

2% and 1% citrate solutions were prepared by dissolving 20 or 10 g of citric acid monohydrated in a litre of distilled water, and adjusting the pH with concentrated NH_4OH. Similarly, 1.2% and 3% EDTA solutions were prepared by dissolving weighed amounts of disodium ethylenediamine-tetracetic acid in distilled water, and adjusting the pH with ammonia.

The ion-exchange resins used in the procedure were Dowex 2X8, 200 to 400 mesh, Dowex 1X8, 100 to 200 mesh, and Dowex 1X8, 200 to 400 mesh, anion-exchange resins, and Dowex 50X-X8, 100 to 200 mesh, cation-exchange resin. Packed resin beds 3 cm in height (approx. 1.5 ml of resin) were prepared and tipped with polyethylene tubing and clamps to control the flow rates so as not to exceed 0.5 ml · $\cdot$ cm^{-2} · min^{-1}. A column containing hydrated antimony pentoxide (HAP) was similarly prepared.

Two groups of mixed standards were also irradiated and processed to allow quantitative measurements, and to check the completeness of recovery. The amounts of the constituents in each group, with their respective decay half-lives and main photopeak energies, are given in Table 1.

Experimental procedures

Heart tissue from adult rats was used as biological tissue samples in the activation and group-separation procedures. The blood-free heart tissue was freeze-dried under high vacuum at liquid nitrogen temperature for 48 hrs, until constant weight was attained. The average weight of the samples was 0.8 g. The samples were then sealed in plastic for irradiations of 4 hrs or less, or in quartz ampoules for longer irradiation times. The irradiations were performed in the vertical core irradiation tube of the RV-1 swimming-pool reactor, at a thermal flux of $7 \cdot 10^{13}$ n · · $cm^{-2} \cdot sec^{-1}$.

After activation the samples were removed from the vials and wet-digested in a silica-coated 50 ml beaker, using 3 ml of conc. HNO_3, 2 ml of 60% $HClO_4$ and 2 ml of 30% H_2O_2. Some Si and Ta was found to be adsorbed on the glass. Dowex 2X8 anion-exchange resin in the bromide form was used to remove all interfering bromine activity at the start of the separation scheme. The resin also retained Cl, I, Ag, Ru, Au, Pt, Os, Ir and Hg. The halogens (Fraction IA) could be subsequently eluted, leaving the metal group (Fraction IB) on the resin.

The first eluent with 0.2% NaBr solution was then evaporated to approximately 0.5 ml, acidified with 3 ml of 12M HCl, and introduced onto a hydrated antimony pentoxide (HAP) column, which retained all Na, Se, Rb and Sb, and any unoxidized As(III), if present (Fraction II). The eluent from the HAP is run directly into a Dowex 1X8 anion-exchange column in its Cl^- form. The chloride non-adsorbable fraction eluted with 12–15 ml of 11–12M HCl was kept for further separation. Further elution with decreasing strength acid solutions separated the following groups: Fraction III, containing Zr and Hf, was eluted with 8 ml of 9M HCl + 0.025M HF; Fraction IV, containing Co, Nb, Ge and W, was eluted with 12 ml of 5M HCl + + 0.5M HF; Fraction V, eluted with 9 ml of 1M HCl, contained Fe, Mo, Ni, Ga, Te and possibly also some Cd. Fraction VI was eluted with 8 ml of 0.01M HCl and contained Zn and also Cd. Sn and Sb (Fraction VII) were retained on the anion-exchange resin.

The first chloride non-adsorbable eluent from the anion-exchange column was evaporated to dryness in a platinum crucible, and dissolved in 2 ml of 2% (pH 3) ammonium citrate. If chromium was thought to be present, the dissolved sample was kept in a water bath at 95 °C for 20 min, with further additions of 2 or 3 0.5 ml portions of the citrate to complete the conversion of Cr(III) to its citrate complex. The solution was then introduced into a Dowex 50W-X8 cation-exchange column in its NH_4^+ form. Fraction VIII, containing P, Sc, Cr and As(V), was eluted with 8 ml of 2% (pH 3) citrate solution, and Fraction IX (Cu, Cd and In) was eluted with 8 ml of 1% (pH 3) HEDTA. Further elution with 8 ml of 1% (pH 5) citrated Fraction X (all the rare earths La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu), 9 ml of 1M KSCN separated Fraction XI (K, Rb, Cs, Re and Mn), and Fraction XII, consisting of Sr, Ba and Ca, was retained on the resin. If desired,

Table 1

COMPONENTS IN MIXED STANDARDS A. Short irradiation times (less than 1 day)

Element	Concentration, μg/ml	Active isotope*	Decay half-life	Main γ-energy, keV	Other useful photopeaks, keV
I	10	^{128}I	25.01 m	443.0	526.4
Cl	100	^{38}Cl	37.2 m	2,167.4	1,642.4
In	100	^{116m}In	54.1 m	1,293.4	417.0, 1,097.1
Ge	10	^{75}Ge	82.8 m	264.7	198.9
Ge	100	^{77}Ge	11.30 h	264.4	211.2, 215.6
Ba	10	^{139}Ba	83.2 m	165.8	–
Nd	30	^{149}Nd	1.73 h	211.4	114.4, 269.6, 423.5
Yb	5	^{177}Yb	1.9 h	150.3	1,079.8, 1,240.9
Yb	5	^{175}Yb	4.19 d	396.1	113.5, 282.6
Dy	0.005	^{165}Dy	2.35 h	94.5	279.3, 361.1, 545.7
Ni	50	^{65}Ni	2.55 h	1,481.7	366.5, 1,115.4
Mn	0.1	^{56}Mn	2.582 h	846.8	1,811.0
Si	200	^{31}Si	2.62 h	1,266.2	–
Sr	100	^{87m}Sr	2.83 h	388.5	–
Cs	2,000	^{134m}Cs	2.90 h	127.4	–
Cd	20	^{117}Cd	3.2 h	273.3	1,576.1, 1,997.2

Cd	50	^{115}Cd	53.5 h	527.8	492.4	
Lu	0.1	^{176}Lu	3.69 h	88.3	–	
Br	1	^{80m}Br	4.42 h	37.0	–	
Br	5	^{82}Br	35.4 h	554.3	619.1,	776.5
Ru	10	^{105}Ru	4.44 h	724.2	469.5,	676.2
Hf	50	^{108}Hf	5.5 h	332.3	215.5,	443.2
Mo	100	^{99m}Tc(n, γ, β^-)	6.007 h	140.5	–	
Mo	100	^{99}Mo	2.78 d	739.8	141.6,	181.0
Er	5	^{171}Er	7.5 h	308.2	116.6,	295.9
Te	50	^{127}Te	9.3 h	417.2	57.6,	360.5
Eu	0.01	^{152m}Eu	9.3 h	841.6	121.8,	963.6
K	20	^{42}K	12.42 h	1,524.7	–	
Cu	3	^{64}Cu	12.75 h	1,345.7	β^+ (511.0)	
Pd	2	^{109}Pd	13.46 h	647.3	311.4,	781.4
Zn	50	^{69m}Zn	13.7 h	438.9	–	
Ga	2	^{72}Ga	14.1 h	834.0	630.0,	2,201.5
Na	2	^{24}Na	15.00 h	1,368.4	2,753.9	
Zr	0.7	^{97}Zr	16.8 h	507.8	355.5,	1,147.8
Re	0.7	^{188}Re	16.8 h	155.0	478.1,	633.1

Table 1 (cont.)

Element	Concentration, μg/ml	Active isotope*	Decay half-life	Main γ-energy, keV	Other useful photopeaks, keV
Ir	0.5	^{194}Ir	17.8 h	328.0	644.6, 938.4
Gd	10	^{159}Gd	18.0 h	363.4	58.0, 226.0
Pr	1	^{142}Pr	19.2 h	1,575.6	-
Pt	50	^{197}Pt	20.0 h	77.5	191.3
W	2	^{187}W	23.9 h	685.7	134.2, 479.4
W	2,000	^{185}W	75.0 d	125.5	-
Sn	0.5	^{121}Sn	26.2 h	no γ	(β^- 0.383)
As	2	^{76}As	26.4 h	559.1	657.1
Ho	0.7	^{166}Ho	26.8 h	80.6	1,379.1, 1,581.5
Os	20	^{193}Os	31.0 h	139.0	460.5, 557.5
Ce	100	^{143}Ce	33.0 h	293.2	664.4, 721.7
Ce	2	^{141}Ce	32.53 d	145.5	-
La	2	^{140}La	40.23 h	1,596.0	328.7, 486.9, 815.8
Sm	1	^{153}Sm	46.8 h	103.2	69.7
Y	15	^{90}Y	64.0 h	1,750.0	-
Hg	15	^{197}Hg	64.1 h	77.6	191.4

Hg	300	^{203}Hg	46.6 h	279.2	–	
Au	1	^{198}Au	2.696 d	411.8	675.9	
Sb	7	^{122}Sb	2.74 d	564.1	692.7,	1,140.6
Sb	700	^{124}Sb	60.2 d	602.7	722.8,	1,690.9
Ca	10^5	^{47}Sc (n, γ, β^-)	3.347 d	160.0	–	
Ca	10^5	^{47}Ca	4.53 d	1,297.0	489.2,	807.9

B. Long irradiation times (over 1 day)

Pt	50	^{197}Pt	20.0 h	77.5	191.3		
W	2	^{187}W	23.9 h	685.7	134.2,	479.4	
W	2,000	^{185}W	75.0 d	125.5	–		
Sn	500	^{121}Sn	26.2 h	no γ	(β^-383)		
As	2	^{76}As	26.4 h	559.1	657.1		
Ho	0.7	^{166}Ho	26.8 h	80.6	1,379.1,	1,581.5	
Os	20	^{193}Os	31.0 h	139.0	460.5,	557.5	
Ce	100	^{143}Ce	33.0 h	293.2	664.4,	721.7	
Ce	2,000	^{141}Ce	32.53 h	145.5	–		
La	2	^{140}La	40.23 h	1,596.0	328.7,	486.9,	815.8
Sm	1	^{153}Sm	46.8 h	103.2	69.7		

Table 1 (cont.)

Element	Concentration, μg/ml	Active isotope*	Decay half-life	Main γ-energy, keV	Other useful photopeaks, keV
Gd	50	^{115}Gd	53.5 h	527.8	492.4
Gd	7,000	^{115m}Gd	44.1 d	934.1	484.9, 1,289.9
Y	15	^{90}Y	64.0 h	1,750.0	–
Hg	15	^{197}Hg	64.1 h	77.6	191.4
Hg	300	^{203}Hg	46.6 d	279.2	–
Au	1	^{198}Au	2.696 d	411.8	675.9
Sb	7	^{122}Sb	2.74 d	564.1	692.7, 1,140.6
Sb	700	^{124}Sb	60.2 d	602.7	722.8, 1,690.9
Ca	10^5	^{47}Se (n, γ, β$^-$)	3.347 d	160.0	–
Ca	$7 \cdot 10^4$	^{47}Ca	4.53 d	1,297.0	489.2, 807.9
Ca	10^5	^{45}Ca	162.7 d	125.0	–
Yb	5	^{175}Yb	4.19 d	396.1	113.5, 282.6
Lu	1	^{177m}Lu	6.71 d	208.3	113.0, 228.5
Nd	50	^{147}Nd	11.06 d	91.2	319.4, 531.0
P	50	^{32}P	14.31 d	no γ	($\bar{}$1.709)
Rb	80	^{86}Rb	18.66 d	1,077.7	–

Cr	8,000	^{51}Cr	27.8	d	320.1	-
Ru	2,000	^{103}Ru	39.8	d	497.1	610.3
Hf	80	^{181}Hf	42.4	d	482.2	133.1, 345.9
Fe	10^5	^{59}Fe	45	d	1,099.0	1,291.5
Fe	$4 \cdot 10^4$	$^{54}Mn(n,p)$	313	d	834.8	-
In	70	^{114m}In	50.0	d	190.0	558.3, 725.2
Sr	$8 \cdot 10^4$	^{89}Sr	50.8	d	909.1	-
Ni	100	$^{58}Co(n,p)$	71.4	d	810.7	(β^+ 511.0)
Tb	20	^{160}Tb	72.4	d	879.3	298.6, 966.0
Ir	4	^{192}Ir	74.3	d	316.5	295.9, 468.0
Sc	20	^{46}Sc	83.82	d	889.3	1,120.4
Ta	50	^{182}Ta	115	d	67.8	100.1, 1,121.2, 1,221.4
Se	50	^{75}Se	120.4	d	136.0	264.7, 279.5
Tm	10^4	^{170}Tm	129	d	84.3	-
Zn	10^5	^{65}Zn	243.7	d	1,115.5	-
Ag	2,000	^{110m}Ag	353	d	657.7	884.6, 937.4
Cs	500	^{134}Cs	2.06	y	604.9	796.4, 570.2
Co	700	^{60}Co	5.258	y	1,173.2	1,332.5
Eu	5	^{152}Eu	14	y	121.8	344.2, 1,407.8

*Produced by (n, γ) reaction, unless otherwise indicated.

Fraction XII could be removed from the resin by elution with 8 ml of 1.2% (pH 6.5) EDTA, giving Fraction XIIA (Ca and Sr), followed by 8 ml of 3% (pH 9) EDTA, separating Fraction XIIB (Ba). Fraction XI could also be further subdivided by introducing the eluent into a Dowex 1X8 anion-exchange column in the SCN^- form, and eluting Fraction XIA (K, Rb and Cs) with 6 ml of 1M KSCN, and Fraction XIB (Re and Mn) with 20–25 ml of water.

Discussion and conclusions

The main advantages of the procedure as developed is the clean separation of the large majority of components in 12 to 15 distinct groups, each containing isotopes having wide ranges of decay half-lives, with no serious overlapping of the gamma-photopeak energies. The large interferences produced by ^{32}P and ^{24}Na are removed in separate groups with a small number of other activities (see Fig. 1). With this procedure there is no need to add inactive carriers, and the separation is quantitative, obviating the need for recovery determination, allowing the use of small volumes and rapid flow rates, and greatly reducing the time for complete separation.

One of the improvements over earlier separation procedures is the complete adsorption of Se on the HAP column, as in earlier separation schemes Se could not be handled satisfactorily. Similarly, As(III), if not completely oxidized in the digestion step, is quantitatively removed in Fraction II, and does not interfere in Fractions III and IV, as was the case previously. Another improvement is the removal of Au, Ag, Hg, and the platinum metals in Fraction IB, before the two evaporation steps, especially if platinum crucibles are used.

Because of the slow rates of interconversion of the various Sb(V) complexes, some of the Sb may not be retained on the Dowex 18X column but will be eluted with the zinc in Fraction VI. Similarly, only about 90% of the Cd is eluted in the second anion-exchange step, and the rest may appear in Fractions V and VI. If Si and Ta are present, their activities can be adsorbed on about 1 ml of silicone-treated fine glass beads, following the digestion step, as both Si and Ta are strongly adsorbed on glass. If Ta is not removed at the beginning of the separation procedure, it can behave very erratically throughout the scheme, and create interferences in many fractions. Similarly Si would be adsorbed on the glass columns. The separation recoveries obtained from mixtures of irradiated standards are given in Table 2.

As concentrated NH_4OH is used as a buffer for the various complexing solutions, he cation-exchange resin must be preconditioned with 5M NH_4Cl, to avoid the displacement of H^+ ions by NH_4^+ ions, with the resultant decrease of the pH, decomposition of the complexes and readsorption of the metals.

There is very little interference between the gamma-energies in each fraction. In Fractions IA, II, III, IV, VI, VII, VIII, XIA, XIB, XIIA and XIIB all energies

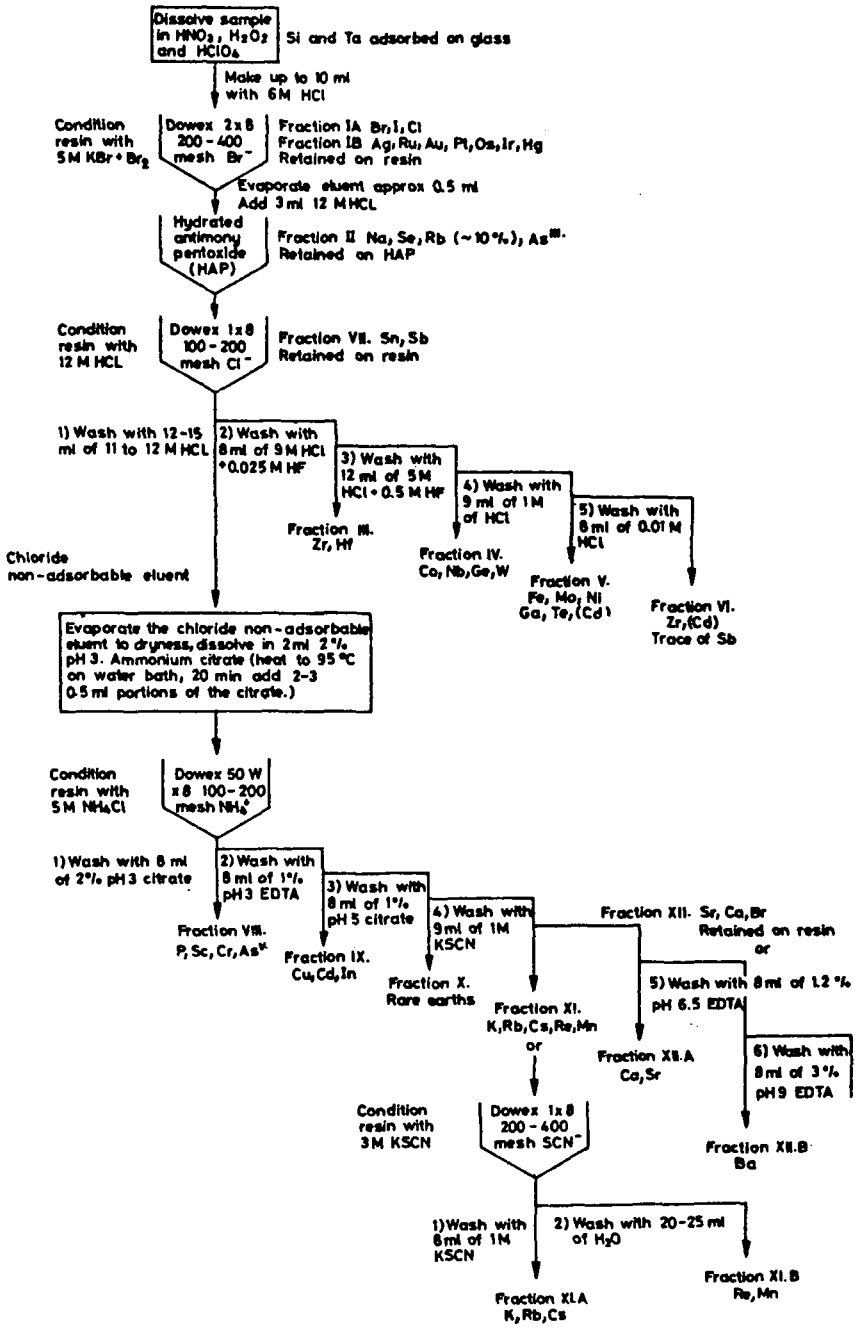

Fig. 1. Ion-exchange group separation scheme

Table 2
Separation recoveries, in %

Elements	Fractions														
	Pre-separation	IA	IB	II	III	IV	V	VI	VII	VIII	IX	X	XIA	XIB	XIIA	XIIB
Si, Ta	100															
Cl, I		~95														
Br		100														
Ag, Ru, Au, Pt, Os, Ir, Hg			100													
Na, Se				100												
Zr, Hf					100											
Co, Ge, W*						100										
Fe, Mo, Ni, Ga, Te							100									
Zn								100 Trace of Se								
Sn, Sb									100							
P, Sc, Cr,* As										100						
Cu, In											100					
Cd							~6	~4			~90					
Rare earths												100				
K, Cs					~5								~95			
Rb					~10								~90			
Mn, Re														100		
Ca, Sr															100	
Ba																100

*The recoveries of Cr and W may sometimes not be complete.

Table 3
Gamma-energies and half-lives of rare earths (Fraction X)

Gamma-energies, keV	Isotope	Half-life	Gamma-energies, keV	Isotope	Half-life
58.0	^{159}Gd	18.0 h	308.2	^{171}Er	7.5 h
69.7	^{153}Sm	46.8 h	319.4	^{147}Nd	11.06 d
80.6	^{166}Ho	26.8 h	328.7	^{140}La	40.23 h
84.3	^{170}Tm	129 d	344.2	^{152}Eu	14 y
88.3	^{176m}Lu	3.69 h	361.1	^{165}Dy	2.35 h
91.2	^{147}Nd	11.06 d	363.4	^{159}Gd	18.0 h
94.5	^{165}Dy	2.35 h	396.1	^{175}Yd	4.19 d
103.2	^{153}Sm	46.8 h	486.9	^{140}La	40.23 h
111.6	^{171}Er	7.5 h	531.0	^{147}Nd	11.06 d
113.0	^{177m}Lu	6.71 d	545.7	^{165}Dy	2.35 h
113.5	^{175}Hb	4.19 d	664.4	^{143}Ce	33.0 h
114.4	^{149}Nd	1.73 h	721.7	^{143}Ce	33.0 h
121.8	^{152m}Eu	9.3 h	815.8	^{140}La	40.23 h
121.8	^{152}Eu	14 y	841.6	^{152m}Eu	9.3 h

Table 3 (cont.)

Gamma-energies, keV	Isotope	Half-life	Gamma-energies, keV	Isotope	Half-life
145.5	^{141}Ce	32.52 d	879.3	^{160}Tb	72.4 d
150.3	^{177}Yb	1.9 h	963.4	^{152m}Eu	9.3 h
2[illegible]3.3	^{177m}Lu	6.71 d	966.0	^{160}Tb	72.4 d
211.4	^{149}Nd	1.73 h	1,079.8	^{177}Yb	1.73 h
226.0	^{159}Gd	18.0 h	1,240.9	^{177}Yb	1.73 h
228.5	^{177m}Lu	6.71 d	1,379.1	^{166}Ho	26.8 h
269.6	^{149}Nd	1.73 h	1,407.8	^{152}Eu	14 y
282.6	^{175}Yb	4.19 d	1,575.6	^{142}Pr	19.2 h
293.2	^{143}Ce	33.0 h	1,581.5	^{166}Ho	26.8 h
295.9	^{171}Er	7.5 h	1,596.0	^{140}La	40.23 h
298.6	^{160}Tb	72.4 d			

differ by at least 10 keV, and in most cases considerably more. In the large Fraction IB, there may be two possible interferences:

(1) The 77.5 and 191.3 keV gamma-photopeaks of the 20 hr ^{197}Pt will interfere with the 77.6 and 191.4 keV peaks of the 64 hr ^{197}Hg, and decay measurements have to be made, if these isotopes are both present, the ^{203}Hg photopeak may be used for confirmation.

(2) The 937.4 keV peak of ^{110m}Ag and the 938.4 keV peak of ^{194}Ir are both minor peaks, besides having widely different decay half-lives, and hence do not present a problem.

Fraction V may present three points of overlap: (1) The 366.5 keV peak of the 2.55 hr ^{65}Ni may interfere with the 360.5 keV peak of ^{127}Te (9.3 hrs), and hence other peaks of these two isotopes must be used. (2) Similarly, the 1.2915 MeV peak of ^{59}Fe (45 d) and the 1.2899 MeV peak of ^{115m}Cd (44.1 d) cannot be differentiated, and hence other gamma-energies must be used if any Cd is present. (3) The 834.8 keV peak of the 313 d ^{54}Mn [from (n, p) reaction with ^{54}Fe] is close to the 834.0 keV peak of the 14.1 hr ^{72}Ga isotope, but the half-lives can be used to separate these. Decay-time differences may also be utilized to separate the activities of the 1.2899 MeV peak of ^{115m}Cd and the 1.2934 MeV peak of ^{116m}In (54.1 min) in Fraction IX.

The rare earths of Fraction X may present most overlaps, (see Table 3), but the isotopes most likely to be present (La, Ce and Sm, and possibly also Dy, Eu, Lu and Ho because of their exceedingly high sensitivities) may be differentiated by their decay half-lives or by use of alternate gamma-photopeaks.

It can be concluded therefore, that the separation is comparatively quick and does not result in serious overlaps, interferences or spread of any components over various fractions.

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