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# RADIOCHEMICAL SCHEME FOR THE DETERMINATION OF MOLYBDENUM AND URANIUM IN BIOLOGICAL MATERIALS BY NAA

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A new universal radiochemical separation scheme for selective and quantitative isolation of molybdenum and neptunium (formed from uranium), from neutron irradiated biological materials has been elaborated. The procedure is based on ion exchange and extraction chromatography with final fixation of molybdenum on a column with α-benzoinoxime supported on Bio-Beads SM2 and neptunium on Dowex 1--X8 [$NO_3^-$]. The separated elements are quantified using gamma-spectrometric measurements. The new NAA method is able to overcome problems associated with high contents of phosphorous in some samples and assures detection limits better than 3 ppb for both elements. The validity of the proposed scheme has been demonstrated by the analysis of several CRM's.

Molybdenum is an element essential for all forms of life.[1-4] Determination of trace amounts of molybdenum especially at very low levels poses some problems and generally Mo is considered to be a "difficult" element.[5,6] This opinion is supported e.g. by the wide range of results obtained for the so called "normal levels" of Mo in human serum.[7,8] Neutron activation analysis (NAA) is known as a technique enabling reliable (i.e. accurate and precise ) determination of many trace elements with very favourable detection limits. In the case of molybdenum, however, one should have in mind that the indicator radionuclide formed in the reaction: $^{98}Mo(n,\gamma)^{99}Mo\text{-}\beta\text{-->}^{99m}Tc$ may also originate from uranium fission : $^{235}U(n,f)^{99}Mo$. According to published calculations,[9] irradiation in a nuclear reactor of 1μg of natural uranium may lead to formation of radiomolybdenum activity equivalent to 0.3-3 μg Mo. So, in the case of materials containing comparable concentrations of both elements this reaction may be the source of significant errors.

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Although several methods for the determination of molybdenum by NAA have been devised, most of them, even from among those recently published,[5,10-17] do not foresee the possibility of simultaneous determination of uranium, which would enable the introduction of an appropriate correction for molybdenum originating from uranium fission.

The purpose of this work was to elaborate a suitable radiochemical separation scheme making possible accurate and sensitive measurement of $^{99}Mo$ - $^{99m}Tc$ activity induced by neutron activation of biological materials, while assuring simultaneously correction of the final result for contribution from the interfering uranium fission reaction.

## Experimental

*Ion exchange resins and adsorbents:* Dowex 50W-X4 [$H^+$] (100-200 mesh) (Serva) was used as received. Dowex 1-X8 [$Cl^-$] (100-200 mesh) (Serva) was conditioned by passing through the column an excess of 1M NaOH, doubly distilled water and 1M $HNO_3$ solutions followed by rinsing with doubly distilled water. Aluminium oxide 90 active acidic (AAO), 0.063-0.200mm (Merck) was used as received.. Bio-Beads SM-2 (20-50 mesh) (Bio Rad Laboratories) were ground and sieved to get fraction of particle size of $0.06mm \leq \phi \leq 0.08mm$ which was purified as described earlier.[18] Column filling for extraction chromatography was prepared by suspending 2g of Bio-Beads SM-2 in a solution of 1g of α-benzoinoxime in acetone. The suspension was heated to ca.$80^0C$ and stirred with a glass rod until the acetone evaporated, and free flowing white powder was obtained .

*Reagents and radioactive tracers:* α-benzoinoxime p.a.(POCh, Poland) was used as received. All reagents and acids were of Analytical Grade. Doubly distilled water from a quartz apparatus was used for preparation of solutions. Radioactive tracers : $^{99}Mo$ - $^{99m}Tc$ (T=66h - 6h), $^{187}W$ (23.9h), $^{24}Na$ (15.0h), $^{46}Sc$ (81.6d), $^{124}Sb$ (60.1d), $^{175,182}Hf$ (70d, 42d), $^{51}Cr$ (27.8d), $^{140}La$ (40.2h), $^{82}Br$ (35.4h) , $^{131,133}Ba$ (11.6d, 10.5y), $^{47}Ca$ - $^{47}Sc$ (4.5d - 3.4d), $^{152,154}Eu$ (13y, 16y) were obtained by irradiating, in Polish reactor EWA, appropriate amounts of nitric acid solutions of these elements prepared from spectrally pure oxides or nitrates (evaporated to dryness in quartz ampoules). Neptunium-239 ( T= 2.35d) was obtained by irradiating, in EWA reactor, an appropriate amount of evaporated solution prepared from $U_3O_8$ uranium chemical standard No 950a (NBS).

*Tracer experiments:* Weight distribution coefficients, $\lambda$ , were determined by batch equilibration. Two to three hundred (200-300)mg of ion exchange resin or the filling for extraction chromatography, was brought in contact (for 24h with occasional shaking) with 10 ml of the solution of desired composition, containing radioactive tracers. After that, the concentration of individual tracer ($A_s$) was determined in an aliquot of the solution by $\gamma$-ray spectrometry and compared with that in the initial solution ($A_o$).
The distribution coefficient was calculated from the relation:

$$\lambda = \frac{(A_o - A_s)}{A_s} \cdot \frac{V}{m_j} \qquad (1)$$

where: $m_j$ -mass of dry ion exchanger or mass of the extractant supported on inert support (g)
$V$ - volume of the solution (ml)

Column separations were performed using glass columns of ca. 0.52cm. I.D. with fritted glass disc or glass wool plug and a stopcock at the bottom. Effluent was collected in fractions and measured with 2"x2" well type NaI(Tl) detector coupled to a scaler or by gamma-ray spectrometry.

*Separation scheme:* Final separation scheme is presented in Fig. 1. The key role play here two systems of coupled columns, consisting of different fillings. The upper column in each of them is designed to act as a guard i.e. to retain radioactive impurities which might interfere with the determination of the analyte. The lower columns, which are of dismountable type, similar to that described in one of our previous works,[19] were designed to quantitatively retain the indicator radionuclides.
The first system (for $^{239}Np$ ) consisted of columns filled with AAO and Dowex 1-X8[$NO_3^-$], respectively, which were washed with 8M $HNO_3$ before use. The second one (for $^{99}Mo$ ) consisted of columns with Dowex 50W-X4[$H^+$] and $\alpha$-benzoinoxime supported on Bio-Beads SM-2 respectively, equilibrated with 0.5M HCl.

*Neutron Activation Analysis:* One to two hundred (100 -200) mg samples of biological certified reference materials (CRMs) were wrapped in aluminium foil of low Mo and U contents, previously washed with concentrated nitric

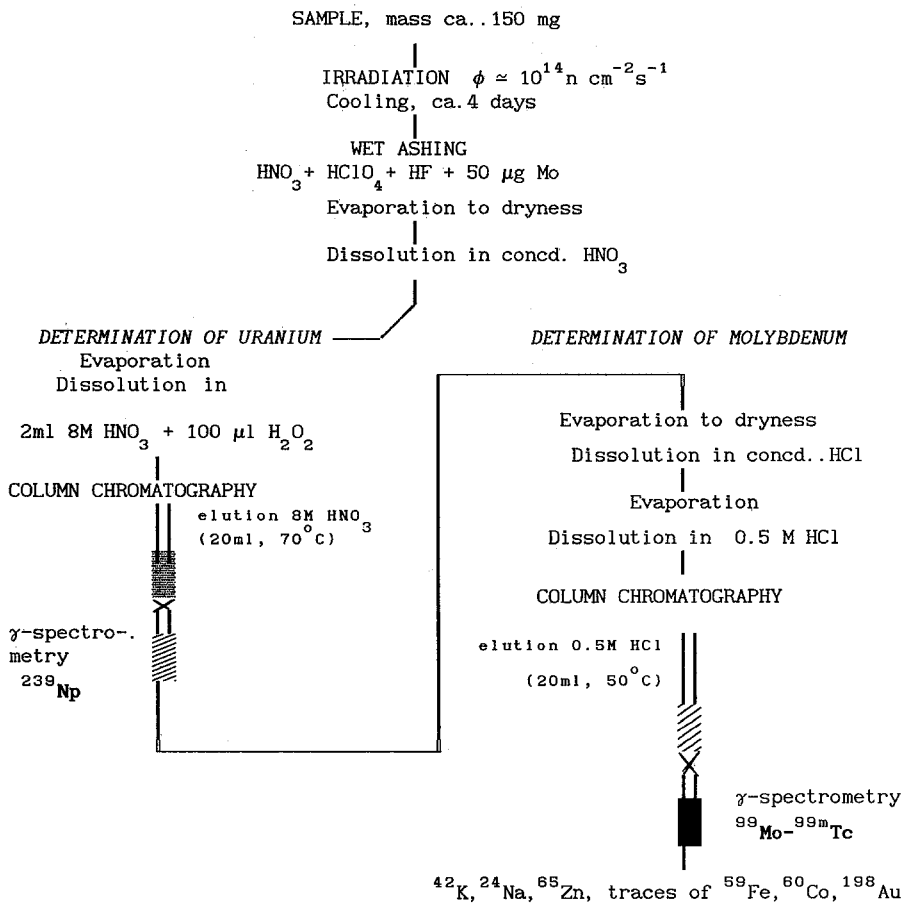


Fig. 1. Radiochemical separation scheme for the determination of molybdenum and uranium in biological materials by NAA

Columns:

Acidic aluminium oxide (AAO), 5 cm x 0.22 cm$^2$ (0.063-0.200 mm); quantitative sorption of $^{32}$P; $^{24}$Na, $^{46,47}$Sc, $^{132}$Te, $^{233}$Pa (partially)

Dowex 1-X8 [$NO_3^-$], 5 cm x 0.22 cm$^2$ (100-200 mesh), **quantitative sorption of Np**

Dowex 50W-X4 [$H^+$], 5 cm x 0.22 cm$^2$ (200-400 mesh); sorption of $^{46,47}$Sc, $^{181}$Hf, $^{131}$Ba, $^{141}$Ce, $^{140}$La, $^{153}$Sm, $^{65}$Zn, $^{198}$Au (partially)

α-benzoinoxime on Bio-Beads SM-2, 2 cm x 0.22 cm$^2$ (0.06-0.08 mm), **quantitative sorption of Mo**

acid and doubly distilled water followed by drying. Molybdenum and uranium standards ( 5μg and 1 μg respectively) were prepared by pipetting appropriate amounts of standard solutions onto thin strips of Al foil followed by drying under an IR lamp. The strips with standards were then folded and placed in Al foil bags. Four (4) samples, 2 standards of Mo, 2 standards of U and a blank (empty Al foil bag) wrappped together in an Al foil were irradiated in the flux of thermal neutrons of ca. $10^{14}$n $cm^{-2}s^{-1}$ for 24 h and cooled for 4 days. After opening, each sample was quantitatively transferred to a conical flask containing 50 μg of Mo carrier and the inner surface of the unfolded Al foil bag was washed with a few ml of concd. $HNO_3$ the washings being added to the sample. The sample was wet-ashed with the mixture of concd. $HNO_3$ + $HClO_4$ ,silica removed by evaporation with HF and the sample was processed, as shown in Fig.1. Neptunium-239 and $^{99}$Mo were quantitatively retained on respective columns. The column fillings with the retained radionuclides were transferred into cylindrical containers and after homogenization were measured by γ-ray spectrometry. The molybdenum fraction was measured after at least 36 h to allow the establishment of $^{99}$Mo - $^{99m}$Tc equilibrium. Molybdenum and uranium standards were dissolved in *aqua regia* and separations as well as γ-spectrometric measurements were performed exactly as in the case of the samples. From these data the apparent Mo content originating from uranium was calculated for each irradiation run. Appropriate corrections could then be introduced into the results of Mo contents in the samples.

Gamma-ray spectrometric measurements were performed with the aid of 213 $cm^3$ HPGe coaxial ORTEC detector (47% relative efficiency) coupled via ORTEC analog line to the multichannel analyser SWAN-3 of the "plug in card" type, cooperating with an IBM/AT microcomputer. The measured detector resolution is 1.9 keV for 1332.5 keV line of $^{60}$Co. Measurements were performed in clock time using pulser technique to account for possible differences in count losses due to dead time and pile up effects although the activities of samples and standards were not high and never exceeded 3000 cps in the whole spectrum. Molybdenum was determined via 140.5 KeV line of $^{99}$Mo - $^{99m}$Tc after establishment of radioactive equilibrium. The determination of uranium via the 277.6 Kev line of $^{239}$Np was employed.

Moisture content of the CRMs was determined according to recommendations of the manufacturers.

## Results and discussion

*General assumptions:* α-benzoinoxime is known to form a stable chelate compound with molybdenum in solutions of mineral acids.[20,21] In an earlier paper from this Laboratory[22], it was shown that molybdenum can be quantitatively retained from 0.1 - 2M HCl solutions on a column with α-benzoinoxime supported on macroporous polystyrene - divinylbenzene copolymer (Bio-Beads SM-2). Such a column will simultaneously separate Mo from most radionuclides present in neutron irradiated biological materials. This method gives good results when applied to the neutron activation determination of Mo in some reference materials of rather high Mo contents (Bowen's Kale, IAEA H-8 Horse Kidney). However, radiochemical purity of the molybdenum fraction is not entirely satisfactory, and in the case of samples of higher uranium contents, one could expect obtaining Mo results which are too high.

The general idea underlying the present work was the desire to construct the separation scheme assuring very selective and quantitative isolation of radiomolybdenum and $^{239}Np$ (for the determination of uranium), respectively, in one run, in order to be able to correct molybdenum results for conceivable errors due to the uranium fission reaction.

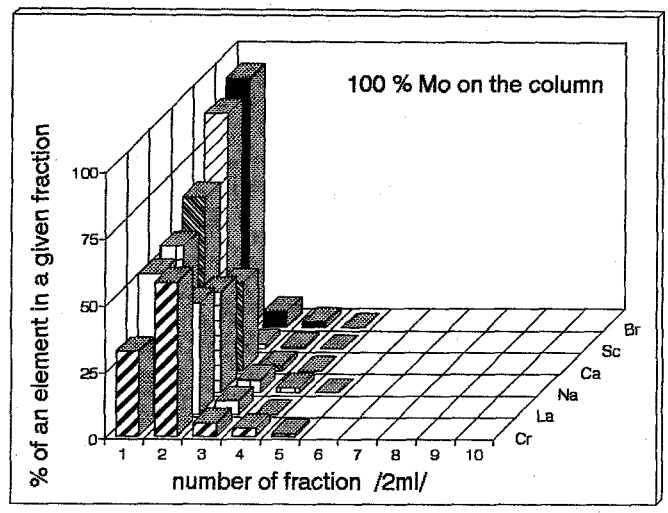


Fig. 2. Separation of molybdenum from several elements by extraction chromatography
Column: 2cm x 0.22cm$^2$ (0.06mm ≤ $\phi$ ≤ 0.08mm); α-Benzoin-oxime on Bio Beads SM-2 (0.5g/g).
Eluent: 1.5M HCl, t=50$^0$C, fraction volume : 2ml, flow rate: 0.5ml/min.

*Molybdenum:* As was shown in numerous tracer experiments as well as when analyzing various biological materials by the method devised previously,[22] molybdenum is quantitatively retained on the column with α-bezoinoxime supported on Bio-Beads SM-2. While most comon elements are rapidly eluted with 0.5-1.5 M HCl solutions (cf. Fig.2), traces of $^{48}Sc$, $^{47}Sc$ (originated from $^{47}Ca$), $^{175,181}Hf$, $^{122,124}Sb$, and $^{198}Au$, were occasionally also found in the molybdenum fraction. Our measurements of distribution coefficients of these elements in the system: Dowex 50W-X4 [$H^+$] - HCl (cf.Fig.3) indicated it should be possible to separate them from molybdenum on a cation exchange column. As can be seen from Fig.4, molybdenum was in fact easily eluted from the cation exchanger with 0.5-1.5 M HCl, while scandium and hafnium stayed on the column. In the final separation scheme 0.5M rather than 1.5M HCl solution was employed as an eluent for the two coupled columns (cf. Fig.1) in order to retain most of the radiocalcium on cation exchange column. This procedure resulted in quantitative isolation of molybdenum of high radiochemical purity with only the trace amounts of $^{47}Sc$ and $^{198}Au$ as the only radioactive impurity.

*Neptunium* Np(IV) shows high affinity to strongly basic anion exchangers in nitric acid medium with maximum distribution coefficient values obtained with $HNO_3$ solutions that are approximately 8M.[23,24] As only a very limited number of elements is uptaken by the resin in these conditions, usually one can get quantitative isolation of neptunium of satisfactory radiochemical purity in a single column procedure, provided all neptunium has been reduced to the tetravalent state. This approach worked reasonably well when analyzing most of biological materials. However , it was found that in the case of samples of high phosphorous content (e.g. NBS 1566 Oyster Tissue), appreciable leakage of neptunium through the column might occur. Moreover, the bremsstrahlung due to $^{32}P$ present in the neptunium fraction adversely influenced the detection limit. In order to make the method fully universal and secure the best possible detection limit, the removal of phosphates is desirable.

According to the literature,[25,26] phosphates can be efficiently sorbed by acidic aluminium oxide (AAO) . Tracer experiments, using the break through technique, revealed that the total sorption capacity of AAO is 5.8 mg $PO_4^{3-}$/g AAO (at $20^0$c in 8M $HNO_3$ medium). Under these conditions neither neptunium nor molybdenum were retained by AAO. Therefore, using 8M $HNO_3$ as an eluent, and coupled columns of AAO and Dowex 1-X8[$NO_3^-$], respectively, it is possible to achieve quantitative retention of neptunium on the anion

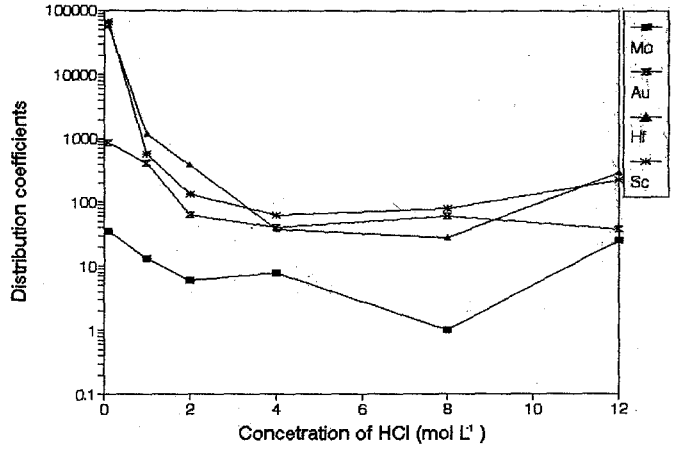


Fig.3. Weight distribution coefficients of several elements in the system: Dowex 50W-X4[$H^+$] - HCl.

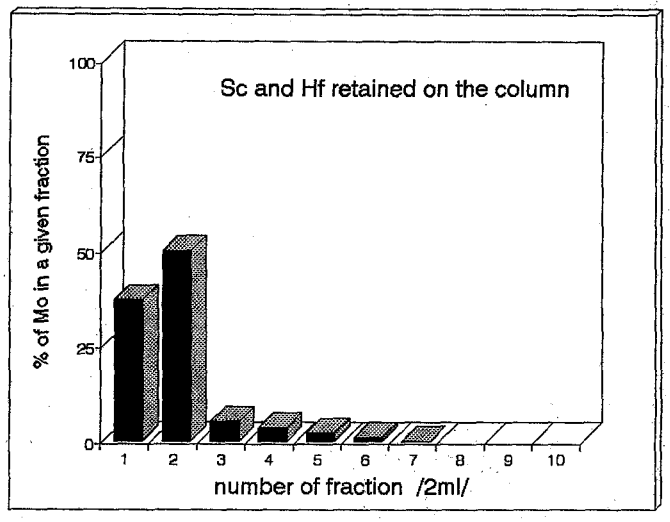


Fig.4. Separation of molybdenum from Sc , Hf and other elements by cation exchange chromatography
Column: 5cm x 0.22cm$^2$ Dowex 50W-X4[$H^+$] (100-200mesh)
Eluent:0.5M HCl, t=50$^0$C, flow rate: 0.5ml/min.

exchange column and at the same time assure good radiochemical purity of the neptunium fraction.

*Recovery and selectivity:* Tracer experiments carried out with the samples of various unirradiated biological materials, and using the separation scheme presented in Fig. 1, proved that the recovery of both Mo and Np in their respective fractions is quantitative (100 ± 2%). Experiments with neutron-irradiated materials confirmed that the radiochemical purity of both molybdenum and neptunium fractions is very good. The scheme is fully universal and can be used for the analysis of all kinds of biological materials both of animal and plant origin.

*Further characteristics of the method:* The detection limits calculated according to Currie's convention.[27,28] amounted to 0.5 ng for molybdenum and 0.1ng for uranium i.e. 2.5 ppb Mo and 0.5 ppb U respectively for 200 mg samples. The correction factor for the interfering uranium fission reaction as determined in our experiments over the time span of 2 years while irradiating samples in various vertical channels of the EWA reactor varied from 0.7 up to 1.5 μg Mo / μg U. Identical correction factors reported in literature are within the range of 0.58 - 3.1 μg Mo / μg U.[29-32]

Hence it is obvious that the determination of the correction factor for each irradiation run , as was practised in this work, is required to achieve the highest accuracy of molybdenum determination.

The only other possible nuclear interference comes from the threshold reaction : $^{102}Ru$ (n, α ) $^{99}Mo$. The cross section of this reaction is small (7μb),[33] and the chances of Ru occurrence in biological materials in concentrations much above ppb levels, are rather improbable. So, this source of potential systematic errors may be neglected.

*Validation of the method* Accuracy of the method was demonstrated by analyzing several CRMs. The results are presented in Tables 1 and 2.

The importance of correcting for the interference, with Mo, due to the uranium fission reaction is evident, for some biological materials, from the data shown in Table 1. There is no doubt that the high "information value" for CTA-OTL-1 comes from the fact that the majority of Mo results were obtained by NAA and the correction for uranium fission was apparently not considered . The same seems to be the reason for the high confidence interval for the certified Mo value in the case of NBS 1571 Orchard Leaves.

Results illustrating the accuracy of the method

Table 1

| Analyzed material | Molybdenum content [ng $g^{-1}$] $/\bar{X} \pm t_{0.05} S \cdot n^{-1/2}(n)/$ | | |
|---|---|---|---|
| | Certified or information value | Result without correction for uranium content | Result with correction for uranium content |
| 1571 NBS Orchard Leaves | * 300 ± 100 | 270 ± 52 (4) | **243 ± 52 (4)** |
| 1547 NIST Peach Leaves | * 60 ± 8 | 75.5 ± 7.0 (4 ) | **57.4 ± 8.1 (4 )** |
| 1566 NIST Oyster Tissue | ** ≤ 200 | 335 ± 50 (4 ) | **204 ± 31 (4 )** |
| Oriental Tobacco Leaves CTA-OTL-1 | ** 390 | 371 ± 39 (6) | **262 ± 38 (6)** |

*- certified value, **- information value

Table 2

| Analyzed material | Uranium content [ng $g^{-1}$] $/\bar{X} \pm t_{0.05} S \cdot n^{-1/2}(n)/$ | |
|---|---|---|
| | Certified or information value | Our results |
| 1571 NBS Orchard Leaves | * 29 ± 5 | **26.7 ± 3.6 (5)** |
| 1547 NIST Peach Leaves | ** 15 | **16.0 ± 2.4 (4)** |
| 1566 NIST Oyster Tissue | * 116 ± 6 | **113.1 ± 3.2 (4)** |
| Oriental Tobacco Leaves CTA-OTL-1 | ** 100 | **103.6 ± 7.8 (6)** |

*- certified value, **- information value

It is worth noting, that failing to determine and correct for this interference is likely one of the reasons for some high results for Mo, published even in recent papers. The examples are: 340 ± 40 ng/g for NBS 1571 Orchard Leaves,[11] 270 ± 9 ng/g in the same material,[15] or 73.7 ± 10.4 ng/g for NIST 1547 Peach Leaves.[14,34]

## Conclusions

The new radiochemical separation scheme devised in this paper makes possible quantitative isolation of radiomolybdenum and neptunium, at high radiochemical purity, from neutron-irradiated biological materials. The use of this scheme for neutron activation analysis, enables determination of molybdenum and uranium in one run down to concentrations of single *ppbs*. The determination of uranium and its fission correction factor against Mo, is a prerequisite for accurate determination of molybdenum in materials containing significant concentrations of uranium.

The proposed method offers an attractive approach to low-level molybdenum determination in biological materials.

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