A DIFFICULT DAY ON THE FILM SET

A group experiment in Pharmaceutical Analysis

Royal Society of Chemistry Analytical Division

North East Regional Heat of the

Schools' Analyst Competition 2007

Analytical Chemistry is all about solving problems. The context could be pharmaceutical analysis such as the experiment that you are doing today, industrial analysis ensuring that an industrial process works efficiently and that the products are of the correct composition, clinical analysis analysing patient samples or environmental monitoring. In all these, analysts have to design experiments, carry them out and interpret the data.

Today's exercise is designed to give you a taste the type of work that an Analytical Chemist has to do. We hope that you find it interesting and challenging and perhaps consider Analytical Chemistry as a career.

First you will have to decide how to tackle your problem, so that you can distribute the workload among your team of three so that each of you is always busy. Then you will need to carry out the experiments, perform the calculations and make some decisions based on the data obtained. A few questions complete the exercise.

You should already have enough background knowledge (but feel free to ask a demonstrator if there is anything that you do not understand), but you will need to show common sense and good organisational skills.

It is part of the RSC Schools' Analyst Competition being carried out in several centres and so we have to operate under the constraints of the competition and keep to time but primarily we hope that you enjoy doing the exercise.

Please read and understand the instructions before commencing and note that there is a strict time limit of three hours for the exercise.

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Health and Safety

This is a practical exercise, so normal rules for safety in the laboratory apply.

Wear laboratory coats and safety spectacles at all times.

Do NOT eat or drink in the laboratory.

Always use the pipette fillers provided, and handle glassware carefully to avoid breakage and cuts.

Keep long hair under control.

IF IN DOUBT ABOUT ANYTHING THEN ASK A DEMONSTRATOR.

.....

THE ORGANISERS THANK YOU FOR PARTICIPATING AND WISH YOU ALL THE BEST FOR YOUR FUTURE.

The Scenario

The well known film actor John Pepp has spent many months rehearsing his role as Jack Marrow in 'Pirates of the Caribbean: Curse of the White Peel'. He has tried to get into the role by living the life of a real pirate as closely as possible. This involves living close to cannon fire, which has given him a constant headache, and also living on a pirate's diet. He has read on the internet that this could lead to a skin condition known as scurvy. Being, in reality, a 21st century person he is prescribed Vitamin C to guard him against the scurvy and aspirin for the headache.

Unfortunately, on the ship, there were a number of other tablets and pellets: Campden tablets for sterilising the barrels for the ship's grog, rat poison for the ships unwelcome furry crew-members, Steeping tablets for rehydrating the stored dried peas. These might have become mixed up and indistinguishable (the blue colouring added to the rat poison to stop such a misfortune had been washed away by the sea-water). John is fairly certain he knew which tablet is which (and hopes he has been taking the correct ones) but has sent them for standard pharmaceutical analysis to keep his mind at rest. At the back of his mind was the worry that the title of the film is really 'Pirates of the Caribean: Curse of the White Pill'!

The competition is analysis of the tablets for aspirin and vitamin C. The supplementary questions ask what would have happened in some of the analyses had he got the tablets mixed up.

Planning

To be successful you will need to plan how each member of the group will use their time. Our estimate of the time required for the experiments is

Experimental	2.0 - 2.5 h each
Calculation	0.5 - 1.0 h each

Decide how you will organise the work within your group; then write out a plan in the form of a flow chart (this is a simple diagram showing the key steps to be taken, in a series of boxes linked by arrows to show the sequence of events). Each box should explain, in brief, the action to be taken at that point. Individual responsibilities should be indicated for each step. The flow chart will be handed in with the results, so do it neatly.

Record the results neatly on the sheets provided, plot your graphs (remember to include titles), perform the calculations then, as a group, draw conclusions from your data. Finally, as a group, answer the questions in the spaces provided.

When you have finished hand in your flow sheet, the result sheets and your graph to the organiser.

Experimental work

Dilution

When making up dilutions, always do so accurately by using a pipette and by making up to a fixed volume in a standardised flask. For example, if you start with a 200 gL^{-1} solution and wish to make up a 10 gL^{-1} solution, you need a 20 fold dilution so you could pipette 5.0 cm^3 of the standard into a 100 cm^3 volumetric flask and then dilute to the mark.

Measurements

Except where you need to run a high concentration first to check that the concentrations will be on scale, you should run your standards in order of increasing concentration to reduce the risk of cross contamination.

Your unknown test samples should have a reading within the calibration range. If this is not the case, then you should normally dilute the sample quantitatively until it gives a signal within the range.

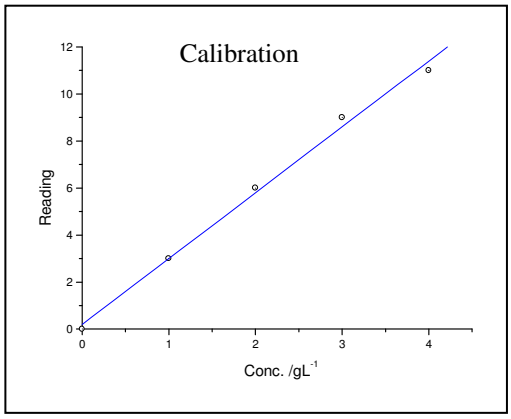
Assessment

For the purpose of the competition, you will be assessed on your analytical results, your presentation of the results and your deductions. The questions at the end are tie-break and will be considered if there are a number of equally good teams.

Treatment of results

Calibration

Draw the points onto clearly labelled graphs. The method here should give a straight line and unless the points are clearly better fitted with a curve, you should estimate by eye the best fit straight line which will be one which minimises the distances from the line to the points rather than the one which passes through the most points.



Calculation

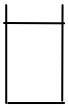
Ensure that your unknown sample gives a reading within the range of your calibration. Dilute the unknown if necessary. Read the concentration off the graph and correct for any dilution before quoting the value in the original sample supplied.

Where requested try to estimate the uncertainty in the answer by quoting the range within which you are reasonably confidant that the answers lies. Suggestions for how to do this are given in the answer book.

1. Ultraviolet spectroscopic determination of acetylsalicylic acid (aspirin) in analgesic tablets

1.1 Introduction

The theory of quantitative spectroscopy is based upon BEER'S LAW which relates to the amount of radiation which is removed from a beam of radiation when it passes through a solution containing an absorbing species. The amount which is removed is termed the amount ABSORBED and is measured in terms of ABSORBANCE. The process may be illustrated as:

Incident beam of radiation →  → beam of transmitted radiation

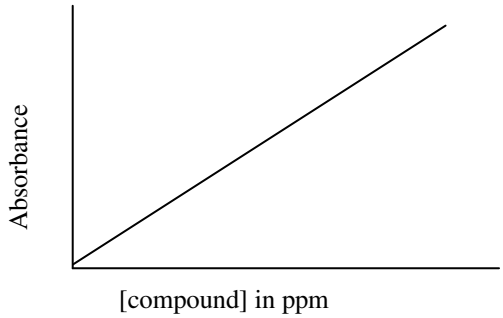
$$\text{Absorbance} = \log \frac{\text{intensity of incident radiation}}{\text{intensity of transmitted radiation}} = abc$$

Where a is a constant related to the absorbing species
b is the path length of the cell containing the species
c is the concentration of the absorbing species

Given that a and b remain constant throughout the analysis:

ABSORBANCE is proportional to CONCENTRATION

Therefore a calibration graph may be produced as illustrated in this experiment



Aspirin absorbs ultraviolet light due to the presence of a benzene ring in the molecule.

1.2 Method

1.2.1 Apparatus

2 x 100 cm^3 conical flask
2 x 100 cm^3, 6 x 50 cm^3 volumetric flasks
funnel
3x 10 cm^3, 1 x 5 cm^3, 1 x 15 cm^3, 1 x 20 cm^3 pipettes
glass rod
cotton wool
Pasteur pipette
Single beam UV spectrometer set up with 50/50 methanol water blank

1.2.2 Reagents

analgesic tablets, 2 per group
50/50 methanol/water made up with HPLC grade methanol
Aspirin standard, 50 mg / 100 cm^3

1.2.3 Procedure

Place two of the analgesic tablets provided in a 100 cm^3 conical flask and dissolve in a small quantity (25-50 cm^3) of the methanol - water (50:50) solvent using an ultrasonic bath with occasional stirring with a glass rod. The ultrasonic agitation should be up to 5 min. The tablet should quickly break up but some of the tablet material will remain undissolved. Quantitatively transfer the solution to a 100 cm^3 volumetric flask and make up to volume with methanol – water (50:50). Mix well. This is solution A. Filter approx. 50 cm^3 of this solution through a small quantity of loosely packed cotton wool into a 100 cm^3 conical flask. Pipette 10 cm^3 of this solution into a 100 cm^3 volumetric flask and make up to volume with methanol – water (50:50). Mix well. Transfer by pipette 10 cm^3 of this diluted filtrate into a clean 50 cm^3 volumetric flask, make up to volume with methanol/deionised water. Mix well. Overall there is a 50x dilution of solution A.

Pipette aliquots 0, 5, 10, 15, 20 cm^3 of the aspirin standard provided into five 50 cm^3 volumetric flasks. Make the flasks up to volume with methanol – water (50:50). The standard solutions now have concentrations of 0,5,10,15,20 mg/100 cm^3.

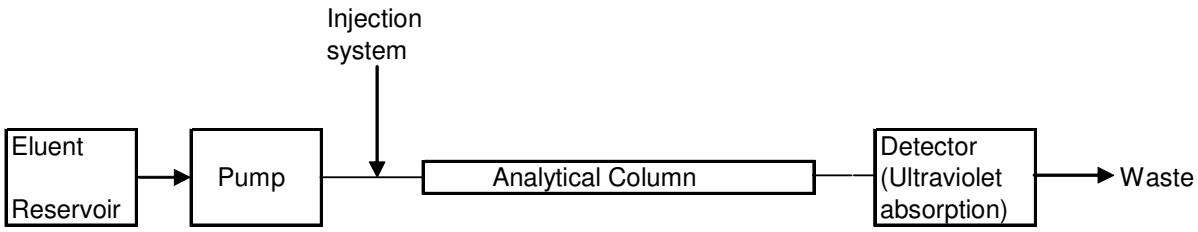
From the UV spectrum of aspirin provided select one wavelength at which to measure the absorbance of the solutions. Measure the absorbances of the standard solutions (repeating to obtain an average value) and then the absorbance obtained from the diluted tablet solution (again repeating). Plot a calibration graph of absorbance vs concentration of aspirin. Use this graph to calculate the weight of aspirin per tablet as mg/tablet.

2.0 Determination of aspirin in analgesic tablets by HPLC

2.1 Introduction

High performance liquid chromatography (HPLC) is a widely used instrumental method used for separating and quantifying components of a mixture. It is widely used in the pharmaceutical industry.

The chromatograph consists of a short column, 3-25 cm in length, packed with a suitable packing usually based on chemically modified silica (eg ODS-silica). A liquid mobile phase is pumped at high pressure, up to 3000psi, through this column. The sample is introduced to the mobile phase stream by means of a sample loop. The method of detection depends on the nature of the sample being analysed; the most widely used detectors are based on the absorption of ultraviolet light. A plot of the detector signal versus time is called a chromatogram. The area under a peak is proportional to the concentration of the component in the solution. The compound is identified by the time it takes for the peak to appear after the injection, each different compound appearing at a different time.



The method used here is a standard addition procedure. A known quantity of a pure aspirin is added to the sample under investigation. Both the sample solution and the sample + standard solution are measured and the increased absorbance in this case is related to the amount of standard added. If W represents the weight of analyte in the unknown sample, and W_s the weight increase due to the addition of the standard, then W may be calculated as follows:

$$W = \frac{A_o . W_s}{A_s - A_o}$$

Where:
A_o and A_s are respectively the peak areas obtained before and after addition of the standard
W_s is the weight of standard added
W is the weight of analyte in the analysis solution.

2.2 Method

2.2.1 Apparatus

Analytical balance capable of weighing 50 mg accurately
High performance liquid chromatograph working in the isocratic mode with a UV detector at 275mm
1x100 cm^3 conical flask
Glass rod
1 x 100 cm^3 volumetric flask
50 cm^3 graduated cylinder
1x funnel + cotton wool
1 x 10 cm^3 pipette
1x 50 cm^3 conical flask
2 x 50 cm^3 volumetric flasks
Syringe for loading HPLC
Pasteur pipette

2.2.2 Reagents

Mobile phase containing water (50 parts) methanol + (50 parts) water
Pure aspirin (acetylsalicylic acid)
Analgesic tablets

2.2.3 Procedure

Place two of the supplied analgesic tablets into a conical flask (100 cm^3), add about 50 cm^3 of the methanol and ultrasonicate for up to 5 min to disperse the tablets. You should stir occasionally with a glass rod. The tablets should quickly break up but some of the tablet filler will remain undissolved giving the final solution a cloudy appearance. Transfer the solution quantitatively into a volumetric flask (100 cm^3). Dilute to volume with distilled water. Mix well. Filter about 50 cm^3 of the solution through a small quantity of loosely packed cotton wool into a 50 cm^3 conical flask and then transfer 10.0 cm^3 aliquots of the tablet solution into two 50 cm^3 volumetric flasks (A and B). Dilute one of these to volume with the methanol/water (Flask A). Mix well.

To Flask B accurately add approximately 50 mg (0.0500g) of pure aspirin and make up to volume with the methanol - water (50:50). Mix well.

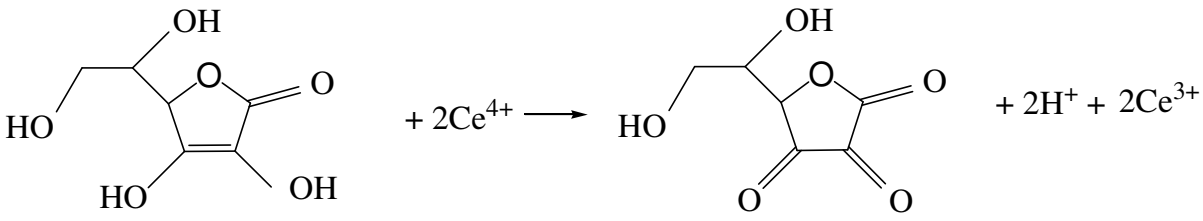
Using the syringe provided, fill the sampling loop of the chromatograph with solution A or B. Activate the Rheodyne valve to introduce the sample onto the HPLC column. When the solute has eluted from the column repeat the process with the other solution.
Analyse each solution twice.

From the peak areas obtained determine the weight of aspirin per tablet.

3.0 Titrimetric determination of vitamin C in vitamin tablets

3.1 Introduction

Ascorbic acid is oxidised to dehydroascorbic acid by Ce^{4+} (Ce = cerium) in acid solution according to the following equation:



Ascorbic acid Dehydroascorbic acid

A titration using an accurately known concentration of Ce^{4+} can determine the amount of vitamin C in a tablet. Ammonium cerium (IV) sulphate , $Ce(SO_4)_2.2(NH_4)_2SO_4.2H_2O$, is used to make up the solution and behaves in solution as Ce^{4+}.

The accurate concentration of Ce^{4+} is determined by a preliminary titration with the primary standard, ferrous ammonium sulphate. Ferrous ammonium sulphate, $(NH_4)_2Fe(SO_4)_2.6H_2O$, has been used to make up the solution and behaves as Fe^{2+}.

Standardisation reaction

$$Ce^{4+} + Fe^{2+} \longrightarrow Ce^{3+} + Fe^{3+}$$

As there is expected to be a variation in the content of the individual tablets the titrations are performed on powder taken from 10 tablets ground together.

3.2 Method

3.2.1 Apparatus

One 25 cm^3 pipette
50 cm^3 burette
25 cm^3 measuring cylinder
2 x 250 cm^3 conical flasks
Mortar and Pestle

3.2.2 Reagents

Ferrous Ammonium Sulphate standard solution (0.1M)
Ammonium cerium sulphate solution approx 0.1M
Ferroin indicator
1 $moldm^{-3}$ sulphuric acid
Vitamin C (ascorbic acid) tablets (10 per team)

3.2.3 Procedure

3.2.3.1 Standardisation of solution of Ce^{4+} solution (approx 0.1M)

Pipette 25 cm^3 of the ferrous ammonium sulphate solution into a 100 cm^3 conical flask and add a few drops of the ferroin indicator. Fill the burette with cerium ammonium sulphate, and titrate to the end point colour change (orange-red to pale blue). Make a note of the accurate concentration of the ferrous ammonium sulphate.

Repeat the standardisation with a second 25 cm^3 of the ferrous ammonium sulphate solution. If concordant results are not obtained (results to within 0.1cm^3) then a third titration may be performed.

3.2.3.2 Analysis of the vitamin tablets

Weigh 10 tablets accurately and powder using a mortar and pestle. Calculate the weight of 3 tablets. Accurately weigh an amount close to the weight of 3 tablets. Dissolve this as completely as possible in a mixture of 30 cm^3 of water and 20 cm^3 of 1M sulphuric acid in a 250 cm^3 conical flask. The solution will still be cloudy due to the other material such as lactose or starch . These compounds are known as excipients. Titrate with approx. 0.1 M ammonium cerium (IV) sulphate using ferroin solution as indicator. Repeat the analysis after weighing out a second portion of powder equivalent to three tablets.

From the results calculate the weight of ascorbic acid in one tablet.

********** *I hope you have all enjoyed the competition. This year we give thanks to the East Anglia region of the Analytical Division of the Royal Society of Chemistry for the original competition and the development of the competition by the Technical and Academic staff at the University of Sunderland.* **********

Results

Name of School or College: _____

Team members _____

_____

_____

Flow chart for experimental design and work allocation:

1. Ultraviolet spectroscopic determination of acetylsalyclic acid (aspirin) in analgesic tablets.

Wavelength chosen =_____ nm

Reason for choice …….

Aspirin concentration mg /100 cm^3.	Absorbance reading 1	Absorbance reading 2	Mean absorbance reading

Test sample			

Concentration of aspirin in final solution (from graph): _____mg/ 100cm^3
(Keep to a realistic number of significant figures.)

Concentration of solution A (ie before 50x dilution) =

= mg/100cm^3

Number of tablets in 100cm^3 solution =

Weight of aspirin in one tablet =

= mg/ tablet

Estimation of uncertainty

Obtain a very rough estimate of the uncertainty in the answer by looking at the distance between the points and the fitted line on your calibration graph. Assume for simplicity that all the error is in the reading. Use the data point which is furthest from the line and convert an error in the reading to an error in the concentration and assume that this will be the worst case uncertainty in the concentration of the answer. The multiply this by the dilution factor.

Uncertainity = ……..

2. Determination of aspirin in analgesic tablets by HPLC

	Area reading 1	Area reading 2	Mean Area
Flask A			(A_o)
Flask B			(A_s)

Weight of aspirin standard added to flask B = g

= mg = W_s

Weight of Aspirin in flask A = $\frac{A_o \times W_s}{A_s - A_o}$ = mg = W

Number of tablets in original 100 cm^3 solution = tablets/100cm^3 = N

This solution is diluted 5x before injection into the HPLC

Number of tablets in diluted solution = N/5 = tablets/100cm^3

= N/10 = tablets/50 cm^3 = Z

W mg aspirin is found in Z tablets

Weight of Aspirin in one tablet = mg

If the suggested dosage of the aspirin tablets is 1-3 tablets every four hours with a maximum of 4 tablets per day what is the maximum daily dosage of aspirin in mg.

…….. mg

3. Titrimetric determination of vitamin C in vitamin tablets

	Sample 1	**Sample 2**	**Sample 3**

Standardisation of Ce^{4+} solution

Titrant:
Final burette reading (cm^3)
Initial burette readingl (cm^3) _____ _____ _____
Volume of titrant used (cm^3)

Average = cm^3

Concentration of ferrous ammonium sulphate = M

Tablet analysis

Accurate weight of 10 tablets = …… g

Average weight of 1 tablet = g
Average weight of 3 tablets = g

Titration

Accurate weight used in titration (i) …… (ii) ……

Final burette reading (cm^3)
Initial burette reading (cm^3) _____ _____
Volume of titrant used (cm^3)

Calculations

(i) Standardisation of Ce^{4+}

If $C_1V_1 = C_2V_2$
Where C_1 = concentration of ferrous ammonium sulphate M
V_1 = volume of ferrous ammonium sulphate used cm^3
C_2 = concentration of Ce^{4+} (unknown) M
V_2 = volume of Ce^{4+} titrated cm^3

Calculate the accurate concentration of Ce^{4+}

Accurate concentration of Ce^{4+} = M

(ii) Calculation of amount of Vitamin C in tablet

	Titration (i)	Titration (ii)	
Titration volume	……	…….	cm^3

Number of moles of Ce^{4+} added to each solution =

= …… ……

Number of moles of vitamin C in flask = …….. ……
(inspect the equation of the reaction)

Number of g of Vitamin C in the flask = …….. …….. g (F)
(M_r Vitamin C = 176)

No of g in an average tablet

= $\frac{F \times \text{Average Weight of 3 tablets}}{3 \times \text{Weight of powder used in titration}}$ = …….. …… g

Average weight of Vitamin C in 1 tablet = g

= mg

Possible errors in analysis

If the daily dose of vitamin C to combat scurvy is 50 mg how many tablets per day is needed to be prescribed?

……. tablets

Summary sheet

Aspirin by Method 1	mg/tablet
Asprin by Method 2	mg/tablet
Vitamin C by Method 3	mg/tablet

Questions

1. In pirate times (17th Century) they would not have vitamin C tablets. How would they have prevented scurvy instead? It is still the best method today for obtaining vitamin C.

2. Cerium is element no 58 in the periodic table as shown overleaf. What is the name of the group of elements which cerium belongs to (elements 58-71)? Hint – There are two common names. You can give either. You might not know one of the names because these elements are not often found in the earth.

3. Steeping tablets contain sodiumhydrogen carbonate, $NaHCO_3$. Describe an easy method which you could test for this.

4. Campden tablets produce sulphur dioxide, SO_2. How would this react with the Ce^{4+}?

5. The rat poison is a complex organic molecule containing a benzene ring. How do you think this would appear in the HPLC chromatogram.

The Periodic Table
0.98 Pauling electronegativity
3 Atomic number
Li Element
6.941 Atomic weight (12C)
1 2.20 2
H He
1.008 4.003
Group 13
Group 14
Group 15
Group 16
Group 17
Group 18
5 2.04 B 10.811
6 2.55 C 12.011
7 3.04 N 14.007
8 3.44 O 15.999
9 3.98 F 18.998
10 Ne 20.179
13 1.61 Al 26.98
14 1.90 Si 28.086
15 2.19 P 30.974
16 2.58 S 32.064
17 3.16 Cl 35.453
18 Ar 39.948
31 1.81 Ga 69.72
32 2.01 Ge 72.59
33 2.18 As 74.922
34 2.55 Se 78.96
35 2.96 Br 79.909
36 Kr 83.80
49 1.78 In 114.82
50 1.96 Sn 118.69
51 2.05 Sb 121.75
52 2.10 Te 127.60
53 2.66 I 126.90
54 Xe 131.30
81 2.04 Tl 204.37
82 2.32 Pb 207.19
83 2.02 Bi 208.98
84 Po (210)
85 At (210)
86 Rn (222)
Group 1
Group 2
Group : 3 4 5 6 7 8 9 10 11 12
d transition elements
3 0.98 Li 6.941
4 1.57 Be 9.012
11 0.93 Na 22.990
12 1.31 Mg 24.305
19 0.82 K 39.102
20 1.00 Ca 40.08
21 Sc 44.956
22 Ti 47.90
23 V 50.941
24 Cr 51.996
25 Mn 54.938
26 Fe 55.847
27 Co 58.933
28 Ni 58.71
29 Cu 63.546
30 Zn 65.37
37 0.82 Rb 85.47
38 0.95 Sr 87.62
39 Y 88.906
40 Zr 91.22
41 Nb 92.906
42 Mo 95.94
43 Tc (99)
44 Ru 101.07
45 Rh 102.91
46 Pd 106.4
47 Ag 107.87
48 Cd 112.40
55 0.79 Cs 132.91
56 0.89 Ba 137.34
57 La 138.91
72 Hf 178.49
73 Ta 180.95
74 W 183.85
75 Re 186.2
76 Os 190.2
77 Ir 192.22
78 Pt 195.09
79 Au 196.97
80 Hg 200.59
87 Fr (223)
88 Ra 226.025
89 Ac 227.0
104 Rf (261)
105 Db (262)
106 Sg (263)
107 Bh
108 Hs
109 Mt
110 Uun
111 Uuu
112 Unb
58 Ce 140.12
59 Pr 140.91
60 Nd 144.24
61 Pm (147)
62 Sm 150.35
63 Eu 151.96
64 Gd 157.25
65 Tb 158.92
66 Dy 162.50
67 Ho 164.93
68 Er 167.26
69 Tm 168.93
70 Yb 173.04
71 Lu 174.97
90 Th 232.04
91 Pa (231)
92 U 238.03
93 Np (237)
94 Pu (242)
95 Am (243)
96 Cm (247)
97 Bk (247)
98 Cf (249)
99 Es (254)
100 Fm (253)
101 Md (253)
102 No (256)
103 Lw (260)