

**Large sample neutron activation analysis avoids representative sub-sampling and sample preparation difficulties**

**An added value for forensic analysis**

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1 **Large sample neutron activation analysis avoids representative sub-sampling and sample**  
2 **preparation difficulties: an added value for forensic analysis.**

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4

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8 **Abstract**

9 A crucial part of any chemical analysis is the degree of representativeness of the measurand(s) in the  
10 test portion for the same measurands in the object, originally collected for investigation. Such an  
11 object usually may have either to be homogenized and sub-sampled, or digested/dissolved. Any of  
12 these steps introduce sampling errors, risk of contamination or loss of the measurand(s). Neutron  
13 (and photon) activation analysis and prompt gamma analysis have the capabilities of analyzing large  
14 objects or samples without the need of any pre-treatment, i.e., intact 'as received', with masses  
15 varying from tens of grams to tens of kilograms, and with any type of (irregular) shape.  
16 The basic concept of neutron activation analysis and prompt gamma analysis are shortly revisited  
17 and the scope of application of the large sample analysis with these technique are elaborated on  
18 with an outlook for use in forensic studies, including the analysis of medicinal products and drugs of  
19 abuse.

20 **Keywords**

21 Homogenization, representativeness, neutron activation analysis, large samples, prompt gamma  
22 analysis, medicinal products, drugs of abuse.

23 **1. Introduction**

24 The interpretation of measurement results requires knowledge of the degree of representativeness  
25 of the measurand in the test portion for the corresponding measurand in the originally collected  
26 material. A sample is denoted to be 'representative' when it can be expected to exhibit the average  
27 properties of the material, environment or population it was taken from [1]. This is a common and  
28 recognized issue in both analytical chemistry and forensic science. A good example is any large  
29 amount of drug of abuse seized by Law Enforcement Agencies [2]. Drugs of abuse can be analyzed to  
30 measure the percentage of the active ingredient or to obtain the elemental profile with the aim to  
31 infer about a possible common source of seized samples [3].

32 Whenever the analysis of large samples is possible, it is much easier to get representative sampling  
33 results e.g., for the analysis of large batches of drugs of abuse. The trace elements of such samples  
34 would be very useful to infer about their possible common source.

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37 The ultimate test portion to be analyzed for element profiling is often much smaller than the amount  
38 of material collected, varying from a few milligrams to a few grams of solids or in the range of a few  
39 mL in the case of liquids. An indication of the typical test portion sizes routinely measured in the  
40 most common analytical techniques is given in Table 1.

41 Table 1: Typical sizes of the test portions handled in several multi-element analysis techniques [4]  
42

Analysis technique	Solid material mass used or prepared to test portion	Volume used as test portion
Atomic Absorption Spectroscopy (AAS)	typically 1 - 2 g dissolved; maximum approximately 10 g	10 - 20 $\mu$ L
Inductively Coupled Plasma Spectroscopy (ICP)	typically 1 - 2 g dissolved; maximum approximately 10 g	Approximately 500 $\mu$ L
X-ray Fluorescence Spectroscopy (XRF)	Typically up to 10 g	
Instrumental Neutron Activation Analysis (INAA)	typically approximately up to 500 mg	1 to 50 mL

43

44 There is even a tendency going to smaller test portions like in solid state atomic absorption  
45 spectrometry, laser-induced breakdown spectrometry, laser-ablation ICP and in total reflection X-Ray  
46 fluorescence spectrometry where microgram amounts are measured.

47 Analysts may be confronted with the necessity of collecting large amounts of material to ensure  
48 representativeness of the population under study. As an example, Ramsey and Boon [5] elaborated  
49 on the occurrence of hot spots of Pb in a contaminated area (which could reflect a forensic  
50 investigation in case of illegal dumping) and concluded that, for reaching a 10 % expanded  
51 uncertainty of the mean of replicates, a minimum mass of 7 kg should be collected (and analyzed).  
52 There are many more such examples published in which, using Ingamell's sampling constant,  
53 indication were obtained that the minimum test portion size to be analyzed should be in order of  
54 several tens of grams up to even tens of kilograms [6-7].

55  
56 An indication of the representativeness may, to some extent, be achieved by replicate sub-sample  
57 analyses assuming sufficient material is available. Another approach is to homogenize the collected  
58 material (both for solids and liquids) or even dissolute solids<sup>2</sup>. Homogenization not only physically  
59 destroys the evidence but additionally introduces the potential risk of contamination or element loss  
60 by incomplete digestion.

61 Solids, and to some extent liquids, can also be analyzed for chemical element composition without  
62 sub-sampling and even without test portion preparations (such as drying, milling, sieving,  
63 homogenizing), thus circumventing the representativeness problems. X-ray fluorescence analysis can  
64 in principle be applied for this if the interest is limited to the composition of the surface layer of  
65 intact materials. Neutron activation analysis (NAA) allows for bulk analysis; NAA is one among the  
66 few analytical techniques<sup>3</sup> in which there are no physical boundaries for the size of this test portion,  
67 and in principle samples of any size (from microgram to multiple kilograms), any physical shape and  
68 state (solid, liquid) can be processed for assessment of its element content within the technique's  
69 analytical capabilities. Analysis of large samples 'as collected', and without further sample  
70 preparation, reduces also the number of sources of error in the procedure (Figure 1).

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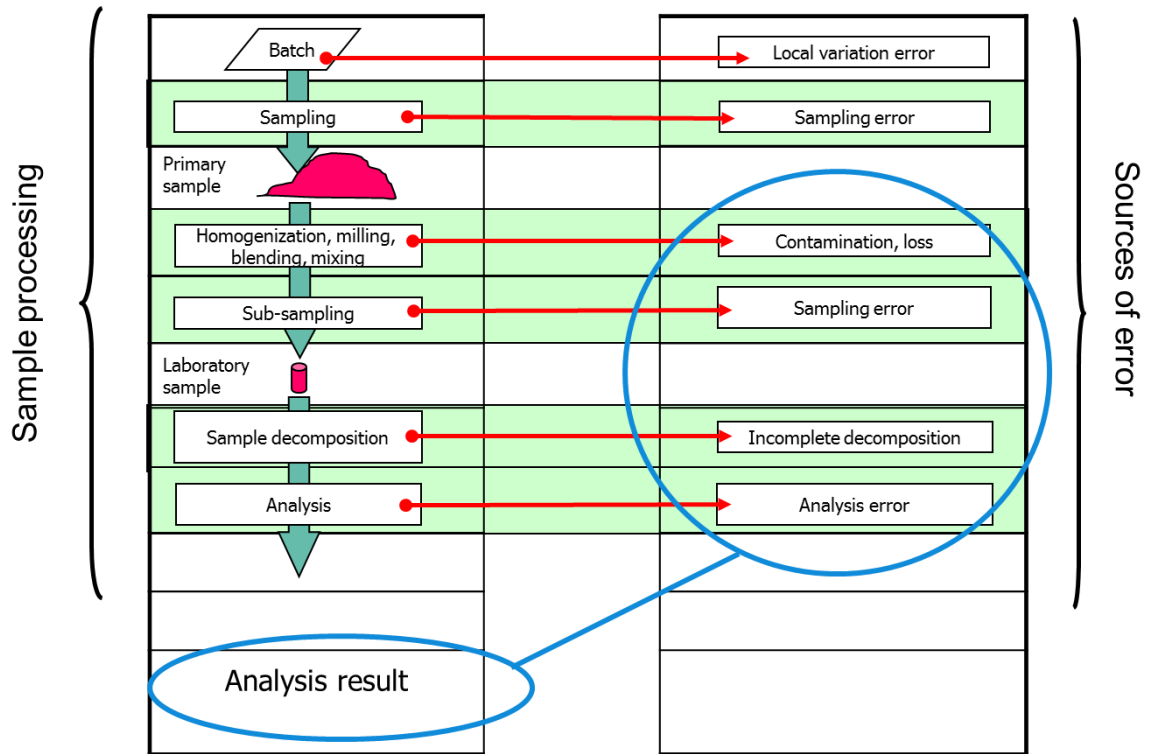
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<sup>2</sup> Homogeneity is defined as 'the degree to which a property or substance is randomly distributed throughout the material' [2].

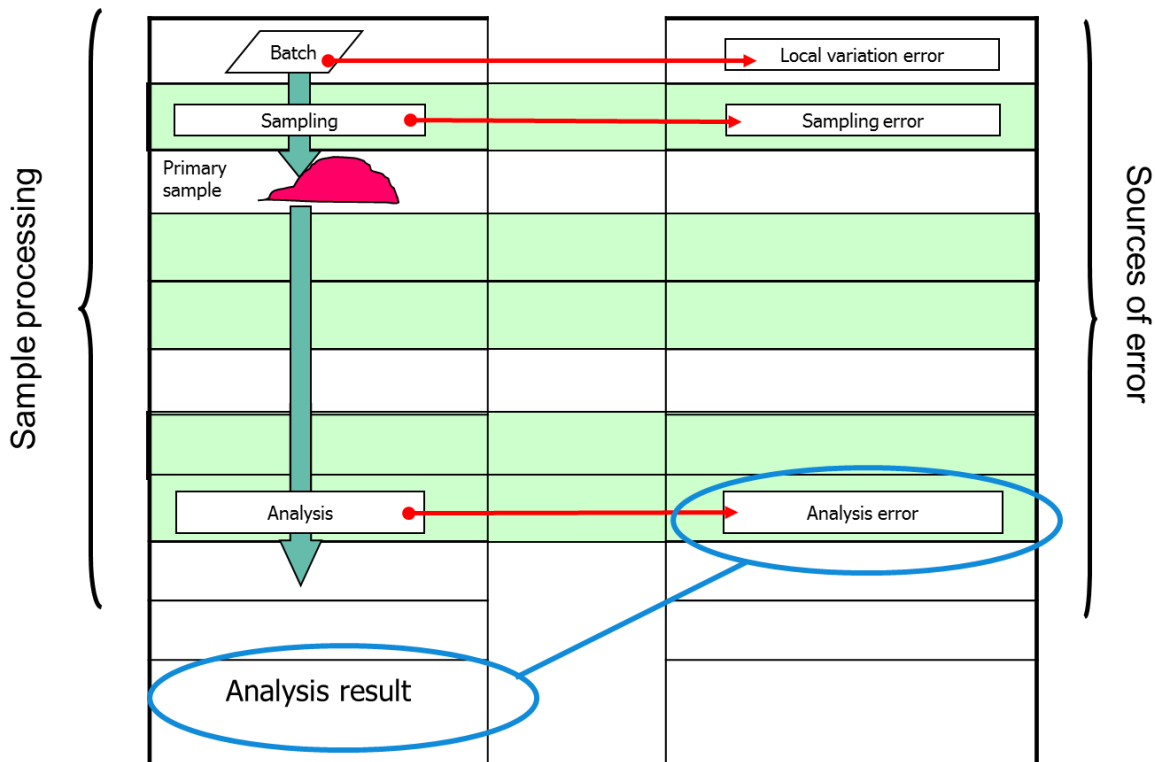
<sup>3</sup> The other techniques are prompt gamma analysis and photon activation analysis [8]. Large sample prompt gamma analysis can equally well be applied using the same neutron source(s) as for neutron activation analysis. The number of facilities for (large sample) photon activation analysis is, however, much smaller than for large sample NAA.



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85 Figure 1. Schematic comparison of potential sources of error during the process from sample  
86 collection to analysis for (top) conventional analysis and (bottom) large sample NAA.

87

88 NAA has already been applied for the analysis of large objects for many decades. The methodology  
89 takes advantage of the high penetrating power of both the incoming radiation for activation  
90 (neutrons) and the outgoing radiation to be measured (gamma-rays). As such, neutron activation  
91 products can be measured in objects with dimensions of several kilograms. Anders and Briden [9]  
92 described the measurement of Oxygen in 60 g steel samples; Kim et.al. described the analysis of 250-  
93 500 mL water samples [10] and many mining and exploration companies use NAA in well-logging  
94 tools [11]. Also, the use of in-vivo NAA for measurement of major, toxic or essential elements in the  
95 human body is an example of NAA's capability to analyze objects having a mass much larger than a  
96 few grams [12]. In the 1990s, following developments by the Delft University of Technology [13-14],  
97 large sample NAA was internationally acknowledged as a unique research reactor based  
98 methodology for analysis of materials under the following constraints:

- 99 - Homogenization of solid materials - to achieve better representativeness' of a small test  
100 portion - is difficult or impossible due to material properties.  
101 - Homogenization is unwanted since it may result in contamination of the material.  
102 - Sub-sampling and/or homogenization is not allowed since the original materials it either too  
103 precious for removal of small pieces or should remain intact.  
104 - Local inhomogeneities in intact materials are subject of study.

105 The principles of this large sample NAA are presented below with an outlook for use in forensic  
106 studies, including the analysis of medicinal products and drugs of abuse.

107

## 108 **2. Large samples analyzed by nuclear analytical techniques**

### 109 *2.1 Principle and characteristics of NAA*

110 Neutron activation analysis is a method for the measurement of the total mass of chemical elements  
111 (in all chemical and physical forms) based upon the conversion of stable nuclei to other, mostly  
112 radioactive nuclei via nuclear reactions with neutrons, and measurement of the reaction products.  
113 The reaction products to be measured are either the radiation, released almost promptly upon  
114 neutron capture ('prompt gamma analysis'<sup>4</sup>) or, if the resulting new nuclei are radioactive, the  
115 radiation emitted during their decay. Gamma-radiation offers the best characteristics for the  
116 selective and simultaneous detection of radionuclides and thus of elements. The activation will result

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<sup>4</sup> Often the term 'prompt gamma activation analysis' is used although the measurement is not based on the induced activity as is done in activation analysis.

117 in a mixture of radionuclides, which can be analyzed by two approaches: (i) the resulting radioactive  
118 sample is decomposed, and chemical separations are applied to obtain fractions with a few elements  
119 each: Destructive or Radiochemical Neutron Activation Analysis; (ii) the resulting radioactive sample  
120 is kept intact, and the elements are determined by taking advantage of the differences in decay rates  
121 by gamma-ray spectrometry at different decay intervals: Non-destructive or Instrumental Neutron  
122 Activation Analysis (INAA). The latter is the most common form of NAA.

123 The most intense source of neutrons for NAA is the nuclear research reactor but also isotopic  
124 neutron sources such as  $^{252}\text{Cf}$  and accelerators serving as neutron generators are used for specific  
125 applications.

126 The metrological basis for NAA was established by the mid-to-late 1990's [15-16], although the  
127 fundamental research was largely completed earlier. In the first decade of the 21st century, it was  
128 demonstrated and internationally accepted that NAA has the potential to fulfil the requirements of a  
129 primary ratio method with evidence on the methods' metrological fundamentals including the  
130 measurement equation, the evaluation and quantitation of all sources of uncertainty and the  
131 metrological traceability of the values of the results [16-17].

132 The analytical characteristics of NAA can be summarized as

- 133 - Real total analysis since the test portion does not have to be dissolved. The size of test  
134 portions in NAA commonly varies from e.g. 5-10 to 200-300 mg.
- 135 - No effects of the chemical or physical state of the measurands as all phenomena (neutron  
136 activation, emission of radiation) are related to properties of the atomic nucleus. There is no  
137 difference whether an element is bound to an inorganic compound or an organic compound,  
138 or if it is present as a pure metal.
- 139 - There is no need for calibrators ('standards') which are fully commutable in chemical  
140 composition with the materials studied; no need for matrix-matching reference materials.  
141 This makes NAA very useful for analysis of materials of complete unknown elemental  
142 compositions.
- 143 - Self-validating properties resulting in a very high degree of accuracy and element specificity.
- 144 - Adequate sensitivity; typically detection limits are in the range of micrograms to nanograms  
145 or even less.
- 146 - Many adjustable experimental parameters for optimization of experimental design.
- 147 - Elements such as H, C, N, and O do not affect the determination of other elements
- 148 - Suitable for measurement of total element mass in the order of micrograms to nanograms or  
149 even less.
- 150 - Less suitable for liquids.
- 151 - Elements like H, C, N, O, Bi, Tl and Pb cannot be measured by NAA.

152 These characteristics make NAA especially suitable - but not limited - for analysis of the following  
153 types of materials:

- 154 - Solid materials difficult to bring completely into a solution, such as from geological origin or  
155 plastics.

- 156 - Solid materials that are easy to contaminate during preparation of the test portion, if e.g.  
157 digestion would be needed for a different analytical technique, such as ultra-pure  
158 substances, ultra-small quantities (e.g. fine dust), biological tissues and body fluids.
- 159 - Solid materials that are unique and should keep their integrity such as from forensic  
160 investigations and/or cultural/historical value.
- 161 - Solid materials of which the bulk composition has to be determined and for which surface  
162 techniques such as XRF and solid-state spectroscopic techniques (e.g. LIBS, laser ablation ICP)  
163 are therefore inadequate.

164 More details can be found in [16].

165

## 166 *2.2 Large sample NAA*

167 NAA is suitable for bulk sample analysis due to the penetrating power of the neutrons and gamma-  
168 rays involved. The intensity of the neutrons is attenuated by interaction with the nuclei of the  
169 material of the test portion as soon as the neutrons enter the test portion; the gamma-rays are  
170 attenuated by interaction with the nuclei of the test portion as soon as they are created and thus  
171 before they leave the sample to be measured. In most NAA procedures, test portions with masses  
172 up to a few hundreds of milligrams are used; for such small amounts, the neutron and gamma-ray  
173 self-attenuation effects may often be insignificant. Moreover, such small test portions can easily be  
174 encapsulated in plastic vials with a well-defined geometry for handling during irradiation and  
175 measurement.

176 A 'large sample' in NAA is defined as a test portion in which these neutron and gamma-ray self-  
177 attenuation cannot be neglected in view of the required degree of accuracy, and/or of which the  
178 physical size requires significant corrections for its deviation from an idealistic point source  
179 geometry, both during irradiation and counting. The geometry may still be well defined, e.g., by  
180 using a 100 mL or 1 L plastic bottle for e.g. granular material, but a major attractiveness of large  
181 sample analysis is that objects of any shape can be analyzed.

182 The corrections for these neutron and gamma-ray self-attenuations can be applied as the related  
183 physics is fully understood [18]. Several approaches (mathematical, empirical) have been developed  
184 to correct for the deviation of the point source geometry [19-20].

185 An important starting point in large sample NAA is that the increase in sample mass from a few  
186 hundreds of milligrams to e.g. tens of grams or even (multiple) kilograms implies that fewer neutrons  
187 are needed for obtaining the same induced activity; the mathematical product of mass and neutron  
188 intensity (neutron flux) should be approximately the same. This also results in almost similar  
189 sensitivities as in normal (small test portion) NAA. As such, large sample NAA can be done not only  
190 with research reactors (and with derived external neutron beams) but also with the isotopic neutron  
191 sources or neutron generators.

192 Calibration and especially quality (trueness) control are still challenging in large sample NAA [21], but  
193 validation has shown that the degree of trueness is well under control.



194 The fundamentals, modes of operation and various opportunities for routine application of large  
195 sample NAA have been reviewed [22].

196

### 197 *2.3 Prompt gamma large sample analysis*

198 Prompt gamma analysis (PGA) is closely related to neutron activation analysis as use is made of  
199 neutron induced nuclear reactions and measurement of gamma-ray spectrometry. The difference  
200 between the techniques is that in PGA the measurement is done simultaneously with the irradiation.  
201 To this end, PGA requires an external neutron beam with a neutron intensity 5-6 orders of magnitude  
202 lower than commonly needed in NAA. The analytical characteristics of PGA are complementary to  
203 those of NAA with respect to the elements that can be measured (such as H and B), sensitivity and  
204 speed of analysis. Because of the external beam, there are fewer constraints in handling large and  
205 irregularly shaped test portions. Moreover, the PGA facility can also be used for large sample NAA by  
206 simply exposing the test portion to the neutrons and subsequently removing it from the beam for the  
207 various measurements, thanks to the intensity of the neutron beam. However, the neutron intensity  
208 may be less optimal for large sample NAA, which causes the activation duration to take much longer  
209 exposing times than the time needed for a PGA irradiation/measurement. Nonetheless, activation in  
210 a neutron beam offers a larger flexibility with respect to the size and shape of objects to be analyzed.

211 Prompt gamma analysis has recently been extended with simultaneous neutron imaging, which  
212 provides an opportunity for 3-dimensional quantitative trace element measurement [23-24].

213

## 214 **3. Opportunities for forensic investigations**

### 215 *3.1 General*

216 Neutron activation analysis applied in forensic investigations for many decades [25]. In 1966 and  
217 1970 topical conferences were held on 'Forensic Activation Analysis'. Several court cases were held,  
218 mostly in the USA, in which NAA results were introduced. An impression of the materials analysed for  
219 such studies is given in Table 2 [25-26].

220 Table2. Examples of materials analysed with NAA for forensic studies

221	Hair, nail clippings
222	Gunshot residues, bullet lead ..JFK case...
223	Paint fragments
224	Glass fragments
225	Soil
226	Grease
227	Drugs (e.g., Marihuana, Heroin)
228	Sweat/Fingerprints
229	Automobile body putty and adhesive tape
230	Moonshine (illegal whiskey)
231	Galvanized wire
232	Paper
233	Diamonds

234 The analyses of the bullet lead fragments from the J.F.Kennedy assassination is perhaps the most  
235 well-spread example of the use of NAA for forensics [27]. Recently, the related analyses were  
236 revisited by Randich and Grant [28], who suggest that the original interpretation of the results is  
237 probably wrong due to overlooking the occurrence of an inhomogeneous distribution of the  
238 elements measured (such as Sb) and subsequent non representative sub-sampling of the test  
239 portions.

240 The role of NAA for forensic studies declined when competitive techniques for elemental analysis  
241 became easier available (such as AAS and ICP) and coincided with the growing interest in the use of  
242 organic and other markers rather than trace elements for characterizing substances.

243 Nonetheless, NAA has attractive analytical characteristics that are widely acknowledged as valuable  
244 complementary to other techniques for elemental analysis. Now, with the availability of large sample  
245 NAA, new opportunities emerge.

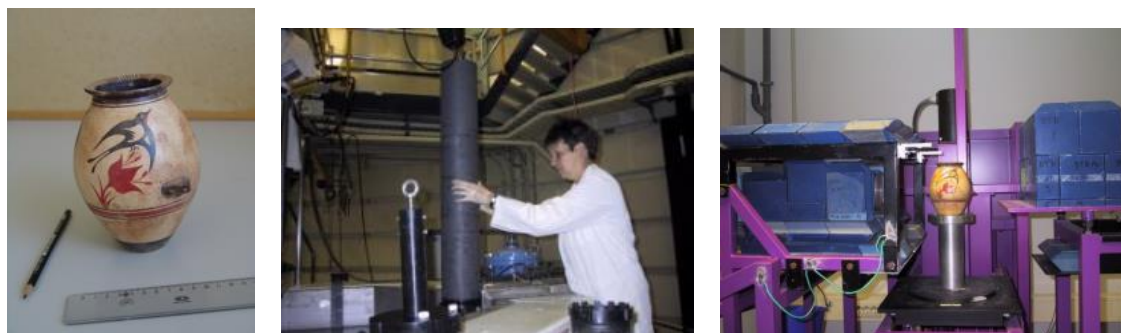
246 Large sample analysis is, as has been outlined in the above, particularly useful for the analysis of

247 • Materials that require thorough homogenization steps -and analytical verification thereof- for  
248 conventional analytical techniques to ensure representativeness of the final (small) test portion  
249 for the bulk sample it originated from. Analyzing an object as received circumvents the various  
250 laborious handling steps with implicit risks of contamination or possible element loss due to, e.g.  
251 incomplete digestion; it eliminates the need for experimental (and in principle indirect)  
252 demonstration of the representativeness - assuming there is sufficient material for preparing at  
253 least 5 replicate test portions to assess the quality of the homogenization.  
254 Soil from (suspected) contaminated areas may serve as an example but also, e.g., street samples  
255 of drugs, fire debris, glass fragments, raw materials for recycling, granular animal fodder and  
256 fertilizer may preferably analyzed in larger quantities than normally processed, e.g. tens of  
257 grams to even kilograms. Entire fragments can be analyzed without the need for pulverizing  
258 them, and neutron beams (with or without prompt gamma analysis) can be used for objects that  
259 do not fit in the regular irradiation facility.

260

261 • Materials which are not allowed to be sub-sampled because they have to remain intact, either  
262 because they are too precious or because of forensic considerations.  
263 Objects related to suspected manipulation or fraud of cultural and archaeological objects fit in  
264 this category. NAA has often been applied to complete bulk analysis of ancient coins (without  
265 sub-sampling) [29]. Recently it was demonstrated – via a mock-up – that entire vases can be  
266 analysed by NAA [30], see Figure 2.

267



268

269 Figure 2. Example of large sample analysis. Left: mock up vase; middle: irradiation container at  
 270 the facility in Delft [11] (which can handle objects up to 100 cm length and 15 cm diameter);  
 271 right: neutron activated vase in the measurement facility [14].

272

273 But also fully machined objects can be analyzed as was demonstrated by Nair et.al. [31] who use  
 274 the signal of an a-priori known amount (mass fraction) of a major component of the material as  
 275 an 'internal standard', thus circumventing all issues such as neutron and gamma-ray self-  
 276 attenuation and the correction for the deviation from the point-source geometry.  
 277 They analyzed complete aluminum cladding tubes, zircaloy plates and steel plugs with masses of  
 278 2.3 g - 67 g, which are construction components of a research reactors.

279

- 280 • Materials that are known to be inhomogeneous and in which the (distribution of the)  
 281 inhomogeneities are subject of study. For such studies, large sample NAA and/or large sample  
 282 gamma analysis can be combined with neutron imaging techniques to identify the position of  
 283 the inhomogeneities and quantify their amounts.

284

### 285 *3.2 The analysis of medicinal products and drugs of abuse*

286 The analysis of the elemental profile of drugs is important for two reasons: on one hand the search of  
 287 elements with potential to be toxic at low doses, on the other hand the profiling of the material, to  
 288 infer about the source.

289 Inorganic impurities are not only important to infer about drug origin, traffic routes, clandestine  
 290 laboratories and methods of drug preparation, but they can be toxic even at low levels, and hence  
 291 should be closely monitored to ensure safety of human health in any product available to the public.  
 292 Since 1990s many scientists tested a lot of technique, including NAA, AAS (Atomic Absorption  
 293 Spectroscopy), ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry) and ICP-MS  
 294 (Inductively Coupled Plasma-Mass Spectrometry) on samples of drugs of abuse. In current years the  
 295 increasing diffusion of fake or illegal pharmaceutical products is requiring an analytical approach  
 296 close to the one developed in cases of drugs of abuse, to protect public health.

297 More than 50 years ago NAA was used to analyse trace element in drugs samples [32], e.g. to obtain  
 298 information on the mass fractions of several toxic elements (Hg, Cd, As, Se, Sb, U and Th) in  
 299 radiopharmaceuticals [33].

300 In a recent review [34] it was reported the trends of the analysis of metal impurities in  
 301 pharmaceuticals products. ICP-MS was proposed in the 2000s to provide rapid, sensitive, precise,  
 302 simple, and element-specific, from semi-quantitative to quantitative alternative to the United States  
 303 Pharmacopeia (USP) and European Pharmacopeia (EP) heavy metals tests for pharmaceutical

304 material [35]. In 2007 ICP-MS was the most used method to find the metal elements in drugs and  
305 pharmaceutical material [36]. ICP-MS today shows high sensitivity, accuracy and precision, and have  
306 the flexibility to handle many other analytical tasks in pharmaceutical production control and  
307 research, but this technique suffers of representativeness issue when applied to illegal products, not  
308 produced with the quality standards of the legal pharmaceutical factories.

309 Recently, LA-ICP-MS (Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry) was proposed  
310 to simplify sample preparation procedures, to use smaller sample size and amount, to minimize  
311 potential polyatomic interferences and to diminish contamination problems compared to ICP-MS  
312 method [37]. This tendency to smaller samples brings a critical issue of representativeness, being  
313 more serious when analyzing products manufactured by illegal production.

314 Dams et al. reviewed [38] in 2001 a variety of analytical techniques for the characterization of street  
315 **heroin** samples, including AAS and ICP-MS. In AAS few elements were found (Zn, Fe). Some years  
316 before, Infante et al. [39] studied 198 illicit **heroin** samples from Andalusia (southern Spain) to  
317 determine the contents of various metals (Cd, Ca, Cu, Fe, Mn and Zn) by AAS. Cadmium and, to a  
318 lesser extent, zinc, copper, and iron, are among the metals detected in heroin that can increase the  
319 inherent toxicity of the drug.

320 ICP-MS was used to analyse 96 illicit heroin samples seized in 2013–2014 to determine 16 inorganic  
321 elements at  $\mu\text{g}/\text{kg}$  (parts-per-billion, ppb) level and to study the clustering outcome by Principal  
322 Component Analysis (PCA) [40]. Bora et al. [41] have analysed 44 illicit heroin samples from  
323 Southeast Anatolia, Turkey by electrothermal AAS (Cd and Pb) and ICP-AES (Al, Ba, Ca, Cu, Fe, Mg,  
324 Mn, Zn). It has been found that the most abundant element was calcium.

325 Zhang et.al. [42] measured fifteen trace elements in illicit heroin by neutron activation analysis. By  
326 statistical analysis it was possible to group the sixty-two analysed heroin samples in two clusters  
327 (Region A and Region B samples) and it was concluded that it is possible to use this method to obtain  
328 information about their geographical origins.

329  
330 Elemental analysis was tested not only on drugs of abuse of natural origin but also on synthetic  
331 drugs. It was determined the trace elements in opium, hashish and ecstasy pills using NAA and  
332 Proton Induced X-ray Emission (PIXE) [43]. PIXE analysis showed that samples contain various  
333 elements including Mg, Al, Si, P, S, Cl, K, Ca, Ti, Fe, Cu, Zn, Rb and Sr.

334  
335 Considering **ecstasy** tablets, the performances of ICP-MS and ICP-AES to analyse the metal elements  
336 were compared on tablets coming from different police seizures in Switzerland [44]. 25 elements  
337 were screened by ICP-AES whereas most of the periodic table was screened by ICP-MS. It was shown  
338 that the ICP-MS is more sensitive than ICP-AES for inorganic analysis of ecstasy tablets. Waddell et al.  
339 [45] used ICP-MS to analyse ecstasy (3,4-Methylenedioxymethamphetamine, **MDMA**) tablets. The  
340 generated data were analysed using different statistical techniques to provide linkage information  
341 from seizure to seizure. Koper et al. [46] described how the elemental analysis can discriminate  
342 **MDMA** powders (57 samples) from illicit production sites and MDMA tablets (97 samples) taken  
343 from large seizures (over 500 tablets) in the Netherlands. Elements mostly present in high  
344 concentration ( $>100 \text{ mg}\cdot\text{kg}^{-1}$ ) were measured with ICP-AES (such as Al, Ca and Mg), elements that are  
345 mostly present in the lower or mid-range concentration range ( $<100 \text{ mg}\cdot\text{kg}^{-1}$ ) were analysed with ICP-  
346 MS. In both techniques the elements Cu, Zn and Pt were measured in very high concentrations.

347 The graphite furnace atomic absorption spectroscopy (GFAA) was tested to analyse **ecstasy tablets**.  
348 Among 6 elements measured in ecstasy tablets (Cu, Mg, Ba, Ni, Cr and Pb) Ba was the only one  
349 offering discrimination between the two ecstasy seizures [47].  
350 Marumo et al. [48] classified seized **methamphetamine** samples in Japan using ICP-MS and AAS to  
351 obtain impurity profiling, providing very useful information on drug intelligence.  
352 ICP-MS was also used to detect metal elements related to two synthetic routes to produce illicit  
353 **methylamphetamine**, Moscow and hypophosphorous [49].  
354 Finally, in 2015 the metal elements in **illicit spice** samples were determined. These are synthetic  
355 cannabinoids (SCs), marketed as legal marijuana alternatives in Europe in the early 2000s. Twenty-  
356 nine samples from street in Ankara (Turkey) were analysed by ICP-MS [50]. In this work, the trace  
357 element contents in the analysed samples were below the limit values determined by the WHO.  
358  
359 Neutron activation analysis is, without doubt, complementary to techniques such as ICP-MS for  
360 measurement of chemical elements in drugs of abuse and associated pharmaceutical products, as is  
361 demonstrated in the few examples reported above. However, there is shockingly little attention paid  
362 in these and other papers to the degree of homogeneity of the samples collected and the  
363 representativeness of test portions. Detection limits, precision and demonstration of degree of  
364 trueness are primarily highlighted. In some papers, the authors reported that material has been  
365 'homogenized', without mentioning the validation thereof. In other cases analysis of replicates is  
366 reported, without providing clarity if these are replicate test portions taken from the (homogenized  
367 ?) sample or replicate analyses of the same test portion. The observed variance -which is relevant  
368 for further interpretation of the data- may be attributed to analytical and sampling errors [51]. The  
369 analytical ability of some techniques (such as ICP-MS) to reach substantial lower detection limits than  
370 before is a valuable asset in the characterization of materials, but at the same time the sampling  
371 error related to the representativeness in the measurement of smaller mass fractions increases.

372  
373  
374

### 375 *3.3 Availability of facilities for large sample analysis and limitations*

376  
377 Facilities for large sample NAA and large sample prompt gamma analysis are already available in  
378 several countries. The International Atomic Energy Agency (IAEA) facilitated a co-ordinated research  
379 project from 2009-2012 on the implementation of research reactor based facilities and  
380 methodologies for the analysis of large sample. This project resulted in operational facilities in Brazil,  
381 Egypt, Ghana, India, Japan (neutron beam based), Malaysia (neutron generator based), Peru (neutron  
382 beam based), Romania, Russian Federation, Syrian Arab Republic, Thailand and USA. Typical object  
383 sizes that can be analyzed vary from volumes of a few cubic centimeters to several tens of liters. A  
384 report on these large sample facilities, the methodologies applied and validation thereof is in  
385 preparation [52].

386  
387 The induced radioactivity in the samples upon exposing the objects with neutrons limits its handling  
388 to authorized radiological workers at locations with a related safety regime. However, the induced  
389 activity decays and the object may be safely released again after a certain period (which may vary

390 from a few days and months up to a year or more) that sometimes can be even well be estimated  
391 before an irradiation is considered.

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393

#### 394 **4. Conclusions**

395 A crucial part in the interpretation of results from chemical analysis is the assessment if the  
396 measured components of the test portion, collected from an object under investigation, are  
397 representative for the components in this object. If the object is large enough, the results from  
398 replicate test portion analyses may indicate this degree of representativeness. There is ample  
399 evidence that the degree of representativeness -at a given degree of confidence- can only be  
400 achieved by analyzing test portions exceeding in size the capabilities of most analytical techniques.  
401 Analysis of such test portions and even the entire object of study can be nowadays carried out by  
402 applying the principles of neutron activation analysis, prompt gamma analysis and photon activation  
403 analysis, without sub-sampling.

404 Large sample neutron activation analysis is a method built on the methodology of 'normal' neutron  
405 activation analysis.

406 Large sample neutron activation analysis is not commonly available and requires access to a nuclear  
407 analytical laboratory with access to the facilities of a nuclear research reactor or other source of  
408 neutrons but the physics of this technique is fully understood and it has been demonstrated that the  
409 degree of trueness and metrological traceability of the values of the measurement can meet the  
410 highest international metrological requirements.

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