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Measurement of the enriched stable isotope ^{58}Fe in iron related disorders-comparison of INAA and MC-ICP-MS

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ABSTRACT

As a safer alternative for the use of radioactive tracers, the enriched stable ^{58}Fe isotope has been introduced in studies of iron metabolism. In this study this isotope is measured with instrumental neutron activation analysis (INAA) in blood samples of patients with iron related disorders and controls after oral ingestion of a ^{58}Fe containing pharmaceutical. Results were compared with those derived from MC-ICP-MS, applied on the same samples, and analytical and practical aspects of the two techniques were compared.

Both techniques showed an increased absorption and incorporation in red blood cells of the ^{58}Fe isotope in iron deficient patients in contrast to the controls. In all individuals results of INAA measurements were in good agreement with those of MC-ICP-MS (|zeta| < 2). Uncertainties in INAA are substantially higher than those achievable by MC-ICP-MS but the INAA technique offers a high specificity and selectivity for iron close to 100%. In contrast to INAA, sample preparation before measurement is very critical in MC-ICP-MS and interferences with ^{58}Ni and ^{54}Cr may hamper the measurement of ^{58}Fe and ^{54}Fe respectively. Since it takes at least five days after irradiation to reduce the activity of interfering radionuclides (mainly ^{24}Na), INAA is a more time consuming procedure; the need of a nuclear reactor facility makes it also less accessible than MC-ICP-MS. Costs are comparable.

Both INAA and MC-ICP-MS are able to adequately measure changes in iron isotope composition in blood when an enriched stable iron isotope is applied in clinical research. Although MC-ICP-MS is more sensitive, is faster and has easier access, in INAA preparative steps before measurement are simpler and there are hardly demands on the kind and size of the samples. This may be relevant working with biomaterials in a clinical setting.

1. Introduction

The absorption and distribution of iron in men can be studied with the use of iron (Fe) isotopes. Radioactive tracers such as ^{59}Fe have side effects and therefore limited application in clinical medicine especially in children and child-bearing women. Enriched stable non-radioactive isotopes of iron have been introduced as an attractive alternative but they can only be measured with isotope-specific techniques such as multi collector-inductively coupled plasma- mass spectrometry (MS-ICP-MS). With this technique natural stable isotopes of iron have been measured in human blood, animal tissues, natural and spiked standards as well as synthetic samples and in samples with organic and inorganic matrices [1–11]. A critical aspect of this technique, however, is the preparation of the samples, which can be complicated, when a variety of biological materials (blood, faeces, tissue biopsies) have to be analyzed in the same experiment and in the case of inhomogeneous distribution of the element of choice. Since instrumental neutron activation analysis (INAA) has the ability to measure low concentrations of isotopes in various biological materials without complicated preparative steps, it seems an attractive alternative or additional technique in clinical iron research. Furthermore in case of inhomogeneous distribution of an element in the material to be analysed, large -sample INAA allows multi-element analysis in samples up to kilograms [12].

In this study INAA has been introduced to measure the enriched stable isotope ^{58}Fe in blood samples of patients with iron-related disorders and healthy controls after oral ingestion of a ^{58}Fe containing

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Table 1
Nuclear properties of ^{58}Fe, ^{56}Fe, ^{54}Fe stable isotopes and their main γ-lines [13].

	Nuclide	Relative isotopic abundance	Nuclear Reaction	Half live of the product	Cross section σ (in b, 10^{-24} cm^{-2})	Activation products (decay/sec per 1 μg element) after one hour irradiation ϕ_{th} = thermal neutron flux ϕ_{epi} = epithermal neutron flux ϕ_f = fast neutron flux	Main γ-lines and between (): gamma-ray emission probabilities (%)
Slaw reactor neutrons	^{58}Fe	0.28	(n, γ)^{59}Fe	44.6 d	1.15 ± 0.02 b	$\phi_{th} = 10^{13}$, $\phi_{epi} = 2.10^{11}$ cm^{-2} sec^{-1} 0.256	1099.3(56), 1291.6(44)
Fast reactor neutrons	^{54}Fe	5.8	(n,p)^{54}Mn	312.5 d	82.5 ± 5 mb	$\phi_f = 10^{12}$ cm^{-2} sec^{-1} 4.77E-3	834.8(100)
	^{56}Fe	91.7	(n,p)^{56}Mn	2.6 h	1.07 ± 0.08 mb	2.49	846.6(99), 1811.2(30), 2112.6(15.5)

pharmaceutical. Since such a pharmaceutical was not commercially available, it had to be prepared for this study and can now be applied in future studies on iron metabolism. The measurement of ^{54}Fe was used as reference for the natural occurring iron. When Fe is irradiated with neutrons, radioactive ^{59}Fe is formed from the natural stable ^{58}Fe, while ^{54}Mn and ^{56}Mn from the natural ^{54}Fe and ^{56}Fe isotopes respectively (see Table 1). ^{59}Fe and ^{54}Mn have long half-lives (312 d and 45 d, respectively), in contrast to ^{56}Mn which has a (relatively) short half-life of 2.6 h. Neutron activation of isotopes in biological materials may result in the production of high activity from radionuclides produced from matrix components such as C1, Na and K. However, these radionuclides all have much shorter half-lives than the long half-life activation products of Fe (^{59}Fe, ^{54}Mn), and their interferences are negligible after a decay period of about 10 days.

In view of further research on the use of enriched stable iron isotopes for studies in clinical medicine, we decided to compare the two isotopic-specific techniques with regard to a number of analytical and practical aspects. To our knowledge, such a comparison of INAA and MC-ICP-MS has not been reported before for this specific case. Analytical aspects include e.g. the degree of trueness and (instrumental and methodological) detection limits while availability of instruments and facilities, preparation of material, time consumption and costs determine the practical aspects.

2. Materials and methods

2.1. Preparation of the enriched ^{58}Fe containing oral iron supplement

The ^{58} Fe enriched oral supplement was prepared at the department of pharmacy of the St. Antonius Hospital, Nieuwegein, The Netherlands. The enriched iron was purchased from Isoflex, San Francisco, USA and contained 92.3% ^{58} Fe (Table 2). A total of 73 mg of ^{58}Fe was mixed with 6.86 mL 0.5 M H_2SO_4 and heated to 53 °C until all iron was dissolved. Fe^{3+} was reduced to Fe^{2+} by adding 219 mg of ascorbic acid and the solution was diluted with deionized water to a final Fe concentration of 1 mg/mL. The solution was deaerated and distributed into vials each containing 5 mL of the iron sulfate solution. The vials were stored at 15 °C and all were used within three weeks after preparation.

2.2. Patients and administration of the Fe supplement

After approval by the medical ethical committee, seven patients of the Meander Medical Centre, Amersfoort, The Netherlands and four healthy controls participated in this study: four individuals were known with iron deficiency anaemia (Hb < 7 mmol/L (< 11 g/dL), serum iron < 7 μmol/L and MCV < 75 fL), and three with hereditary hemochromatosis (C282Y mutation) under treatment with blood-letting. The medical history of each person was recorded, as well as eating pattern and smoking status. Some of the demographic data as well as haemoglobin concentration and iron parameters are presented in Table 3. Following overnight fasting (8–9 hour), all subjects attended the hospital where 10 ml blood was collected from each of them and separated in two tubes. One sample allowed for the measurement of the person's full blood count and serum ferritin, transferrin and haemoglobin (Hb) in the hospital. The second sample served for measuring the iron

Table 2
Enriched Stable Isotope Composition of Iron used in this experiment.

Stable isotope	Natural abundance (atom. %)	Available Enrichment (atom. %)
^{54}Fe	5.845	< 0.01
^{56}Fe	91.754	1.0
^{57}Fe	2.119	6.7
^{58}Fe	0.282	92.3

Table 3
Demographic data, haemoglobin (Hb) concentration and iron parameters of the individuals participating in the study.

No	Diagnosis	Sex	Age (years)	Weight (kg)	Length (m)	Hb (mmol/L)	Serum Fe (μmol/L	Ferritin (μg/L)	Trf. (g/L)	Trf. Sat. (%)
1	IDA	F	64	61	1.70	5.9	5	130	2.4	9
2	IDA	M	52	85	1.89	6.8	4	37	2.5	10
3	IDA	F	52	112	1.52	6.8	6	10	3.0	8
4	IDA	M	67	98	1.79	6.2	4	21	2.8	6
5	HH (homozygous)	M	66	107	1.88	8.4	21	312	1.8	49
6	HH (homozygous)	F	42	58	1.67	9.0	24	181	1.8	56
7	HH (homozygous)	M	46	105	1.90	9.2	24	41	2.2	46
8	Contr.	M	64	88	1.82	10,2	20	208	2.0	42
9	Contr.	F	28	64	1.68	9.1	16	24	3.9	17
10	Contr.	M	27	80	1.83	9.5	22	70	2.6	36
11	Contr.	F	27	63	1.62	8.7	19	27	2.7	29

F: female, M: male, IDA: iron deficiency anaemia, HH: hereditary hemochromatosis, Contr.: control, Trf: transferrin, Trf. Sat.: transferrin saturation.

isotopic ratios before the start of the supplementing.

Each person was given 5 mL of the supplement containing 5 mg of enriched ^{58}Fe (92.3%) for oral intake. To obtain a better absorption, the supplement was mixed with 100 mL orange juice. Intake of food, tea or coffee was not allowed for the next 2 h. After two weeks (to allow the incorporation of iron into the erythrocytes) [14] another 10 mL blood was collected. All blood samples were frozen and sent to the Reactor Institute, Delft, the Netherlands for analyses.

2.3. INAA

2.3.1. Sample preparation, irradiation and measurement

The blood samples were freeze-dried in an EZ- dry freeze drier (MNL-036-A) from FTS System Inc., Stone Ridge, New York, USA. About 200 mg of each dried sample was weighed and packed in high purity polyethylene capsules. The irradiation took place in the 2 MW nuclear research reactor of the Reactor Institute Delft, Delft University of Technology, The Netherlands. Zinc was used as neutron flux monitor [15] during the irradiations. Each batch of samples irradiated contained also a similarly prepared blank (empty capsule) and a sample of the Standard Reference Material “Bovine liver” (NIST 1577c); both serving for internal quality and trueness control. The irradiation duration was 10 h at a thermal neutron flux of ˜ $4.5*10^{12}$ cm $^{-2}$ s^{-1}. Samples were allowed to decay for two weeks, thereby effectively eliminating all activity from the short half-life radionuclides such as ^{24}Na in order to improve the detection limits. The induced radioactivity was measured during 3 h using a well-type Ge detector from ORTEC, USA, with an absolute photopeak efficiency of 11% for the 1332 keV photopeak of ^{60}Co and 13% for the 1099 keV photopeak of ^{59}Fe. The gamma-ray spectra were analysed using the APOLLO software [15].

The principle of measuring the ^{59}Fe/^{54}Mn mole fraction ratios by INAA is as follows:

- When irradiated with neutrons, radioactive ^{59}Fe is formed from both the natural stable ^{58}Fe (present for 0.3% in natural iron) and the supplemented isotopically enriched ^{58}Fe. However, radioactive ^{54}Mn is also formed from the natural Fe isotope ^{54}Fe (present for 6% in natural iron) following the reaction ^{54}Fe(n,p)^{54}Mn. We firstly measured the ^{59}Fe/^{54}Mn activity ratio from natural iron only.
- After supplementing isotopically enriched stable ^{58}Fe (now present for 92.3% in iron), blood samples are collected for neutron activation. The measured ^{54}Mn activity indicates the pathway of the natural present Fe, and the measured ^{59}Fe activity, together with the earlier determined activity ratio, make it possible to estimate which fraction originates from the supplemented iron; and thus the efficacy of the supplementation.

(n, gamma) beta, gamma-Ray
^{58}Fe ——————> ^{59}Fe (T1/2 = 45 days) ——————————> ^{59}Co

(n, P) gamma -Ray
^{54}Fe ——————————————> ^{54}Mn (T1/2 = 312 days)——————— ^{54}Cr

2.4. MC-ICP-MS

2.4.1. Sample preparation

Sample preparation was performed in the Reactor Institute Delft (RID) of the Delft University of Technology following a procedure provided by Van Heghe et.al [7]. It consists of two main steps: sample digestion and isolation of iron fractions using a chromatographic purification. All acids used were of ultrapure quality prepared in a polyethylene volumetric flask with ultrapure water from Milli-Q system (Advantage A10). Nitric acid (HNO_3 65%) and hydrochloric acid (HCl 30%) were Suprapur (Merck KGaA, Darmstadt, Germany). Hydrogen peroxide (H_2O_2 30%) was of VWR International BVBA, Leuven Belgium. All lab ware used (bottles, vials, pipet tips etc.) were cleaned very well first with diluted nitric acid and secondly by Milli-Q water. For the digestion 7 mL of nitric acid (HNO_3) (14 M) was added to 2 mL of the whole blood sample. This mixture was heated to 110 °C for 14 h in a closed vessel. After cooling, the digested sample was dried down at 90 °C. The residue was redissolved in 1 mL of (HCl (8 M) + 0.001% H_2O_2). One hour before the separation 50 μL of H_2O_2 was added to make sure that Fe is present in its highest oxidation state.

The chromatographic separation was performed using a Bio-Rad Poly-Prep column as described in literature [7].

To check (and validate) the separation protocol using a synthetic blood sample, a digested sample of NIST Standard Reference Material 1577c “Bovine Liver”, (containing 1 mg of Fe) was used. The eluted elements were collected in 1 mL fractions. First the matrix element were eluted and then Fe fractions. The iron fractions were measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) to check for the presence of other matrix elements. A Perkin Elmer Optima 4300DV (ICP-OES) was used. The instrumentation setting and parameters of the ICP-OES are shown in Table 4.

Table 4
Instrumentation parameters and settings (ICP-OES).

Parameter	Settings
Power:	1300 W
Plasma gas flow:	15 L/min
Auxiliary gas flow:	0.2 L/min
Nebulizer gas glow	0.8 L/min
View distance	15 mm
Sample flow rate:	1.5 ml/min
Wash rate	1.5 ml/min
Wash time	30 sec
Wavelength	Fe 259.9, Ca 393.4 Na 588.9, Ni 231.6 Cr 267.7, Co 228.6

Table 5
Results of the Fe elute for Bovine Liver (mg/L) measured with ICP-OES.

Elution Step → Elements↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ca	0.269	0.134	0.103	0.070	0.035	0.042	0.007	0.005	0.025	0.057	0.038	0.034	0.005	0.003
Fe	< DL	57.141	20.612	20.597	14.150	7.037	3.414	1.852	1.250	0.920	0.630	0.429	0.243	0.204

DL = detection limit, instrumental DL (Ca = 0.0005 mg/L, Fe = 0.005 mg/L).

2.4.2. Analytical validation

A series of Fe standard solution used for calibration was prepared by diluting the stock solution of 1000 mg/L Fe (Iron ICP standard). The calibration of iron was found to be linear with correlation coefficients above 0.99.

The ICP-OES results are shown in Table 5. It was found that the solution contained no (by ICP-OES) measurable concentrations of other elements such as Na, Ni, Cr and Co. The column efficiency for isolation of Fe was 90%. There was a small amount of Ca, but fortunately this did not interfere with the measurement of Fe.

2.4.3. MC-ICP-MS analysis

MC-ICP-MS analysis was performed at the Faculty of Earth and Life Sciences of the VU University, Amsterdam, the Netherlands. Before analysis by MC-ICP-MS, all samples were checked again for any traces of Cr and Ni by ICP-MS (Thermo Finnigan X Series II) of the same faculty. Instrumentation setting and parameters are shown in Table 6. Because the detection limits of the ICP-MS are lower than from ICP-OES, still small amounts of Cr and Ni were detected at µg/kg level.

The instrument used for the $^{58}Fe/^{54}Fe$ isotope ratio measurement was a Thermo Finnigan MC-ICP-MS (Neptune), operating in high resolution mode (R > 9000 5%–95%) using typical operating conditions adjusted on a per day basis for optimum sensitivity and peak shape. The sample introduction system used was a Scott type-double pass spray chamber combination equipped with a PFA-ST auto-aspirating nebulizer. Instrumentation setting and parameters are shown in Tables 7 and 8.

The standard- sample bracketing technique (SSB) was used to correct for the mass bias in the MC-ICP-MS. Samples were analysed using IRMM-014 Fe (from EU Institute for Reference Materials and Measurements, Geel Belgium) as bracketing standard [16], bracketed every sample and intensity matched. Analysis were done on 300 µg/L solution An in-house Fe solution was used as a (long term) internal laboratory standard. It was prepared by diluting 1000 mg/L ICP iron standard (Merck, ICP standard solution, Darmstadt Germany) in 1% HNO_3 (prepared from sub-boiling double distilled suprapure HNO_3, Merck, Darmstadt, Germany). Long term Fe data for in-house at the time: $\delta 56$ = -0.50274 +/- 0.4247 (2 SD.); n > 100, timespan > 5 year.

2.5. Ethics and statistics

The study protocol was approved by the Medical Research Ethics Committee United (MEC-U), Nieuwegein, The Netherlands as well as by the Medical Ethics Committee and board of directors of the Meander Medical Centre, The Netherlands. All participants gave informed consent. Where relevant, statistical analysis was performed using SPSS (IBM Statistics 20).

Table 6
Instrumentation parameters and settings (ICP-MS).

Parameter	Setting
Plasma power (W)	1400
Nebulizer gas flow rate (L/min)	0.9
Auxiliary gas flow rate (L/min)	0.9
Plasma gas flow rate (L/min)	16
Integration	3 x 0.2 sec
Scan mode	Continues
Read delay (s)	120 nsec
Sample time (sec)	90

Table 7
Instrumentation parameters and settings (MC-ICP-MS).

Parameter	Setting
Sample uptake rate	100 µL/min
Sample gas flow rate	0.9 L/min
Effective sample uptake rate	~ 85 µL/min
RF power	1200 W
Plasma gas flow rate	14 L/min
Auxiliary gas flow rate	0.9 L/min
Number of ratios analysed	90
Integration time	4.196 sec.

Table 8
Detector configuration.

L4	L3	L2	L1	C	H1	H2	H3	H4
Not used	–	^{54}Fe	Not used	^{56}Fe	^{57}Fe	^{58}Fe	^{59}Co	–
–	^{53}Cr	^{54}Cr	–	–	–	^{58}Ni	–	^{60}Ni

3. Results and discussion

3.1. INAA results

The INAA calibration was verified by measuring the ratio of the iron mass fractions for iron of natural isotopic composition in human whole blood (n = 12) and the Standard Reference Material Bovine Liver (NIST 1577c) (n = 3). Calculated on the basis of the 1099 keV peak of ^{59}Fe and the 835 keV of ^{54}Mn and at 'perfect' calibrations, this ratio should be 1.00. The experiments resulted in an average value peak area ratio of 1.04 ± 0.04 for the human whole blood and 1.02 ± 0.03 for the SRM (NIST 1577c) which values are in statistical agreement with the idealistic value of this peak area ratio. The small bias could be attributed to small differences in the neutron energy distribution in the irradiation facility at the time of calibration and at the time of this experiment.

The natural mole fraction ratio of $^{58}Fe/^{54}Fe$ is 0.0482 ± 0.0005. An increase in this ratio is due to the absorbed fraction of enriched ^{58}Fe administered. The measurable difference in the mole fraction ratio between before and after intake depends on the uncertainties in the measurement results before and after intake, as well as on the degree of confidence that a difference exist. If we want to state with a 99% degree of confidence that there is a difference, the mole fraction ratio MFR_{after} after uptake can be estimated from the mole fraction ratio before intake, MFR_{before}, by

$MFR_{after} > MFR_{before} + 3^* \sqrt{\{(unc_{after})^2 + (unc_{before})^2\}}$ and $unc_{before, after}$ = the combined standard uncertainty of the mole fraction ratios before and after uptake, respectively.

In the INAA results, the mole fraction before intake is 0.0480 +/- 0.001; assuming a similar value for the (combined standard) uncertainty after intake, the mole fraction ratio after intake should exceed the value of 0.052 for a 99% confidence verification of an increase to the normal value.

Table 9
Results of the $^{58}Fe/^{54}Fe$ mole fraction ratios before and after intake of enriched ^{58}Fe using MC-ICP-MS compared to INAA (1–4 anaemic, 5–7 Hemochromatosis, 8–11 controls).

Patient No.	Before intake			After intake			Δ% excess (INAA result)
	MC-ICP-MS 1st run No correction for Cr and Ni	MC-ICP-MS 2ed run With correction for Cr and Ni	INAA	MC-ICP-MS 1st run No correction for Cr and Ni	MC-ICP-MS 2ed run With correction for Cr and Ni	INAA	
1	0.0470	0.0470	0.0480	0.0820	0.0820	0.0860	79.20
2	0.0440	0.0470	0.0470	0.0620	0.0620	0.0619	31.70
3	0.0480	0.0480	0.0481	0.0470	0.0650	0.0680	41.40
4	0.0477	0.0477	0.0480	0.0440	0.0570	0.0580	20.8
5	0.0477	0.0480	0.0472	0.0480	0.0490	0.0489	3.60
6	0.0478	0.0480	0.0481	0.0480	0.0480	0.0482	0.21
7	0.0478	0.0480	0.0470	0.0480	0.0510	0.0480	2.13
8	–	–	–	0.0480	0.0480	0.0482	0.00
9	–	–	–	0.0480	0.0510	0.0532	10.37
10	–	–	–	0.0480	0.0480	0.0488	1.24
11	–	–	–	0.0476	0.0510	0.0531	10.17

For the MC-ICP-MS results, a similar calculation indicates that a difference can be observed at 99% certainty if the fraction ratio exceeds the value of 0.0481.

The percent enrichment or (Δ% excess) is a term used in iron absorption studies to express measurement of enrichment relative to base line ratio (at time = 0) [14]; Unless there was a previous isotope administration, the baseline measurement should reflect the natural abundance ratio. It is calculated according the following equation:

$$\Delta\%\ excess\ = \frac{58Fe\text{: }54Fe\ (enriched) - 58Fe\text{: }54Fe\,(baseline)}{58Fe\text{: }54Fe\,(baseline)}\ X\ 100$$

The Δ% excess proved to be high in all anaemic patients (patients 1–4) compared to the hemochromatosis (Patients 5–7) and the healthy group (8–11) (Table 9) which means that the blood is significantly enriched in ^{58}Fe and therefore reflects their ability to absorb iron and incorporate it into blood within 2 weeks. For the control group the t = 0 was not determined because unintentionally the blood was not collected before intake of enriched ^{58}Fe, hence the natural ratio was assumed.

An expected increase of iron absorption could not be observed in the INAA results of the samples collected from the hemochromatosis patients [17–19]. The exact reason is not known. Since iron is easily stored in organs and tissues in this disorder, it cannot be excluded that all the ingested ^{58}Fe is already deposited there before its uptake by the bone marrow. In this experiment we measured the iron 14 days after ingestion and mainly in red blood cells, so such a mechanism cannot be excluded. Measurements more shortly after ingestion may provide adequate information in this type of patients. Since the absence of an increased iron absorption was also measured with MC-ICP-MS, this finding cannot be attributed to INAA as measurement technique (see Table 9). Further studies are necessary to elucidate this finding. The two female controls, both having regular menses, and therefore a very mild form of iron deficiency, also showed some increase in blood Fe levels two weeks after the tracer was given (see the Δ% excess, Table 9).

3.2. MC-ICP-MS results

Using MC-ICP-MS, the mole fraction ration measured via the internal standard was in good agreement for $^{58}Fe/^{54}Fe$ mole fraction ratio of 0.048 in the first run (No correction for Cr and Ni), reflecting the natural mole fraction ratio. However, the result of this ratio in blood samples was vacillating. For some samples, the $^{58}Fe/^{54}Fe$ mole fraction ratio before intake was higher than after intake of the enriched ^{58}Fe. This could be attributed to the presence of small amounts of Cr and Ni in all samples although a chromatography separation was done. The quality of the separation was initially verified using ICP-OES, and no remaining Cr and Ni could be quantified. However, an additional analysis using ICP-MS –with which much lower detection limits can be attained than with ICP-OES- confirmed the presence of these elements at the μg/kg level. As such, and applying correction for the Cr and Ni interference, the MC-ICP-MS results were in close agreement with the INAA results (Table 10 and Fig. 1). Since ^{53}Cr and ^{60}Ni were measured with every ratio measured, it was possible to correct for the contribution of ^{54}Cr on the ^{54}Fe signal (using the natural $^{54}Cr/^{53}Cr = 0.248421$) and ^{58}Ni on the measured ^{58}Fe (using the natural $^{58}Ni/^{60}Ni = 2.599237$) [20].

The results (see Table 9) show higher values for the mole fraction ratio in blood of anaemic patients after intake than before intake. In hemochromatosis group no difference between before and after intake and the healthy group slightly absorbed iron depending on their body needs); this result is largely equivalent to what can be concluded from the INAA results (Table 9), especially taking into account that (see above) the minimum detectable increase in the molar fraction ratio is significantly higher or INAA than for MC-ICP-MS.

The degree of equivalence in the results of $^{58}Fe/^{54}Fe$ mole fraction ratios of the 7 patients (1–7) after intake of enriched ^{58}Fe using INAA and MC-ICP-MS (after correction (2nd run)) are shown in Fig. 1.

Although accurate results can also be produced by MC-ICP-MS, the accuracy for mole fraction ratio measurements can be influenced by several effects [10,21]. The mass discrimination effect -which can occur due to the space charge effect- plays an important role in mass spectrometry. In the step of ion extraction (during the analysis), the light ions are deflected more than the heavy ions. Other limitations on isotope ratio measurement are the matrix effects which can be overcome with chromatographic purification; and the isotopic fractionation effects of the iron isotopes. The latter was, at this stage, not yet a subject of this study –which was primarily focusing at the major analytical and practical differences between the two techniques- but will be evaluated in our follow-up work. However, the here presented study demonstrates

Table 10
Results of $^{58}Fe/^{54}Fe$ mole fraction ratios of the 7 patients after intake of enriched ^{58}Fe using MC-ICP-MS (only 2nd run presented) and INAA.

No.	MC-ICP-MS $^{58}Fe/^{54}Fe$ mole fraction ratios ± absolute measurement uncertainties (one standard deviation)	INAA $^{58}Fe/^{54}Fe$ mole fraction ratios ± absolute measurement uncertainties (one standard deviation)	Zeta score
1	0.0820 ± 0.000024	0.0860 ± 0.0027	1.48
2	0.0620 ± 0.000025	0.0619 ± 0.0016	0.06
3	0.0650 ± 0.000026	0.0680 ± 0.0018	1.67
4	0.0570 ± 0.000028	0.0580 ± 0.0045	0.22
5	0.0490 ± 0.000025	0.0489 ± 0.0010	0.10
6	0.0480 ± 0.000024	0.0482 ± 0.0005	0.40
7	0.0500 ± 0.000026	0.0480 ± 0.0010	2.00

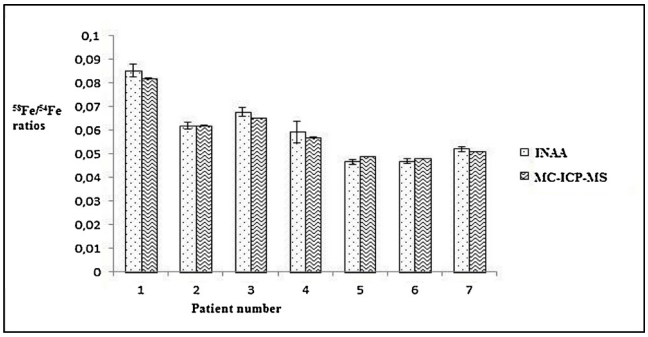

Fig. 1. Results of $^{58}Fe/^{54}Fe$ mole fraction ratios of the 7 patients after intake of enriched ^{58}Fe using INAA as well as MC-ICP-MS (only 2nd run presented). Error bars are related to one measurement.

that the quality of the separation is crucial and needs to be closely monitored to obtain the required level of separation.

The uncertainty of ratios determined by MC-ICP-MS depends on the measurement conditions and the measurement protocol (integration time and the number of measured ratios/ replicates) [10]. It is similarly based on the identification of the contribution of each analytical parameter occurring during the measurement. As shown in Fig. 1 and Table 10, the uncertainties in INAA are substantially higher than those achievable by MC-ICP-MS. Within the laboratory's quality control criteria [22], the calculation of zeta score with ($|zeta| < 2$) indicates good results (Table 10). That means the measurements results of INAA are statistically in agreement with the MC-ICP-MS results.

On the other hand, INAA is the only analytical technique of which objective evidence exists that it can meet the requirements of a primary method of analysis. All sources that contribute to its uncertainty are known and can be quantified [23]. The limitations to the degree of trueness depend on the quality of calibration. This is one of the considerations that INAA is highly appreciated in the characterization of candidate reference materials.

The question which technique has to be used is hard to answer, and depends on several factors including the clinical research question. MC-ICP-MS is very sensitive and easier available than INAA. However, the preparation of material for measurement by MC-ICP-MS is more critical and demanding and, as mentioned in the above, isotopic fractionation cannot always be excluded. Other motives for selection either of them come into play such as costs and time consumption. In terms of analysis costs, a comparison of 'commercial' tariffs for INAA and MC-ICP-MS measurements indicated not much difference. However, the access to, and turnaround time of MC-ICP-MS measurements may often be shorter than of INAA since this technique needs a nuclear reactor facility and it takes at least two weeks before the actual measurement takes place. A such, INAA will mostly be addressed as a method of choice in case an alternative is needed or additive information is crucial.

4. Conclusions

A good agreement was observed between most MC-ICP-MS and INAA measurement results for $^{58}Fe/^{54}Fe$ ($|zeta| < 2$) and therefore both techniques are able to measure changes in iron isotope compositions in blood when an enriched stable iron isotope is applied in clinical research. In one case (patient 7) the zeta score equals 2 and the techniques are not conclusive; from the INAA results no difference in the fraction ratio is found whereas the MC-ICP-MS results indicate an increase. This is not in agreement with the medical status of this patient, haemochromatosis. The uncertainty propagation for MC-ICP-MS covers the most relevant sources. However, an uncertainty source, as e.g. the estimation of Fe isotope fractionation during separation procedure was not quantified in details within this study. For the sake of completeness such information on Fe isotope fractionation during the matrix separation of blood is given in [24]. Unfortunately, no repetitive measurements due to shortage of sample material could be done to assess the precision of these measurements. The critical and most time consuming part of MC-ICP-MS concerns the preparation of materials prior to measuring when not performing Fe isotopic analysis on a routine basis. In INAA, most of the time is consumed by the decay time to effectively eliminate all activity from the short half-life radionuclides such as ^{24}Na.

Although the magnetic sector mass analyser of the MC-ICP-MS works for achieving a high-precision mole fraction ratio measurement, the high mass resolution makes it impossible to separate ions of the same nominal mass to charge ratio by such mass analyser. Therefore an extra step of a chemical chromatography separation was needed to overcome this problem. It has been shown in this study that very high demands have to be set to the effectiveness of such a separation and removal of the elements Cr and Ni interfering with the Fe mole faction ratio measurement. The MC-ICP-MS technique is very sensitive, but some effects on the accuracy of the measurement are very difficult or barely possible to estimate such as those related to the chemical matrix effects when the quality and efficiency of the chromatographic separation are not under control. A good chromatographic separation is not only necessary but it is an integral part of the method.

In contrast, the contributions to uncertainty in INAA are all well-known and can be quantified. In case of measuring iron isotopes, the uncertainties in MC-ICP-MS are much smaller than those achievable by INAA but the INAA technique offers a high specificity and selectivity for iron close to 100% based on the individual characteristics of the induced radionuclides. Its greatest advantage is that the preparative steps before measurement are simple. Its drawbacks however are the availability of a research nuclear reactor and the production of radioactive rest material.

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