Procedimento di determinazione del mercurio organico in prodotti alimentari

<

DOCUMENTO RISERVATO

Allegato a)

RICHIESTA DI BREVETTAZIONE

L'INVENTORE/GLI INVENTORI, AI SENSI DEL REGOLAMENTO DEI BREVETTI E DELLA PROPRIETA' INTELLETTUALE DELL'UNIVERSITA' DI TORINO E DELLE DISPOSIZIONI IN ESSO CONTENUTE, DICHIARA / DICHIARANO QUANTO SEGUE:

1. GENERALITA' DEL PROPONENTE:

NOME E COGNOME…Agnese Giacomino……..

DIPARTIMENTO/CENTRO……Dipartimento di Scienza e Tecnologia del Farmaco...

TEL…0116705679……...

FAX……...

E-MAIL…agnese.giacomino@unito.it…

2. TITOLO DELL'INVENZIONE:

Simple and rapid system for the simultaneous determination of mercury and methylmercury in fish products

3. DESCRIZIONE DELL'INVENZIONE:

(segnalandone le caratteristiche principali, il problema risolto, i vantaggi rispetto ai prodotti esistenti nella stessa area di applicazione, lo stato di avanzamento del progetto, etc.)

Introduction

Mercury is a highly toxic element even when present at ultra-trace levels. It is one of the metals most involved in bioconcentration and biomagnification phenomena along the food chain. It is present in marine ecosystems and, consequently, in fish, which are the main source, for men, of intake of mercury and its methylated more toxic form, namely methylmercury. From the 1950s to the present, mercury absorption through fish has been steadily increasing throughout the world, sometimes reaching values far above the safety limits, especially for some more sensitive categories, such as children.

It is therefore evident how important it is to constantly monitor the levels of total mercury and methylmercury in the fish products placed on the market. Because of the difficulties of

conducting continuous speciation studies (to distinguish between inorganic and methylated forms of mercury) and the lack of databases in this regard, the legal limits refer to total mercury (Hg_{TOT}). The quantification of inorganic mercury (Hg_{IN}) and of methylmercury (Me_{Hg}) is often obtained from total mercury by a conversion coefficient, different for each category of fish species, thus leading, to a mere estimation of the concentration of the two forms.

State of art. Official method

Currently, the official method for the determination of mercury in fish products is the direct mercury analyzer (DMA).

Advantages: the DMA automatically performs the thermal decomposition of the sample, the catalytic reduction of bivalent mercury to elemental mercury, the melting of the latter on a gold trap, its subsequent desorption and the detection of the concentration by spectroscopy in short times (from 5 to 7 minutes) and allows rapid analysis of solid and liquid samples without the need to perform pretreatments and add reagents.

The method then allows, once the instrument has been calibrated, to analyze numerous samples in a short time (12 samples/hour); moreover the quantification limit is very low (LOQ: 0.037 mg/kg of fresh fish).

Disadvantages: the instrument is not portable and is specific for this analyte, it requires qualified personnel, the use of gas, high temperatures, has high purchase and management costs. Furthermore, if the DMA allows the direct analysis of the samples, without any pre-treatment for the determination of total mercury, the determination of MeHg is possible, but requires long times and the use of organic solvents.

The method for the determination of MeHg with DMA is based, in fact, on a double liquid-liquid extraction, first with organic solvent and subsequently with cysteine. The obtained solution is then introduced into the DMA and analyzed. Specifically, the sample preparation procedure involves several steps: 1) Leave all the glassware used for this procedure immersed in 10% (V/V) HNO_3 for 24 hours; 2) weigh 0.7 - 0.8 g of fresh fish sample in a 50 mL Falcon tube. In the case of lyophilized samples weigh 0.2 g of sample and add 0.5 mL of ultrapure water. 3) add 10 mL of hydrobromic acid and stir manually; 4) add 20 mL of toluene and vigorously shake with vortex for at least 2 minutes; 5) centrifuge for 10 minutes at 3000 rpm; 6) if after centrifugation, an emulsion phase between the two phases is present, , break it down by slightly tapping the test tube against the laboratory bench and proceed with a new centrifugation; 7) withdraw about 15 mL from the overlying organic phase and transfer it into a 50 mL Falcon tube containing 6.0 mL of 1% L-cysteine solution; 8) add about 15 mL of toluene into the initial centrifuge tube and repeat a second extraction with the organic phase; 9) after centrifugation, withdraw the remaining upper organic phase and insert it in the

previous 50 mL tube with the cysteine solution; 10) shake vigorously with vortex for at least 2 minutes and centrifuge for 10 minutes at 3000 rpm; 11) withdraw an aliquot of 2-3 mL from the lower phase with a Pasteur pipette, taking care not to drag toluene, and transfer it into a glass vial with a stopper (for example vials for chromatography).

State of the art. Determination of mercury by electrochemical methods

For years our research group has been working on the development of voltammetric methods for the determination of total mercury with benchtop voltammetric analyzers consisting of a potentiostat and a voltammetric stand equipped with an electrolytic cell (Figure 1) consisting of 3 electrodes: a working electrode (WE), an Ag/AgCl/KCl reference electrode (RE) and an auxiliary electrode (AE) consisting of a platinum bar.

Figura 1. Electrolytic cell in a conventional voltammetric analyzer.

For this purpose, both commercial solid gold electrodes (SGEs) and nanostructured gold electrodes (Gold nanoparticle-modified glassy carbon electrodes, AuNPs-GCEs),which allowed to achieve higher sensitivities (10 ng/L), were used as WE. Briefly, the voltammetric determination of mercury relies on a deposition step at the working electrode at 0 V (in which mercury at the oxidation state +2 (Hg^{2+}) is reduced to the elemental form (Hg^{0}) and forms amalgam with the gold present on the electrode surface) under stirring for a short time (typically 60-120 s) and a subsequent stripping step which involves an anodic potential scan from 0 to 0.75 V, during which the mercury is reoxidised and gives rise to a current whose intensity is proportional to its concentration. All experimental parameters had been optimized. Reference samples (with known and certified mercury concentration) very different from each other (soils, atmospheric particulate matter, plants, drugs, ...) previously mineralized in a microwave oven, were analyzed using this technique which had shown high accuracy and precision for all the matrices considered. Figure 2 shows, as an example, the voltammograms obtained from the analysis of a canned tuna sample (canned tuna, CT) as such and after the addition of two aliquots of analyte at known concentrations (each at 2 µg/L).

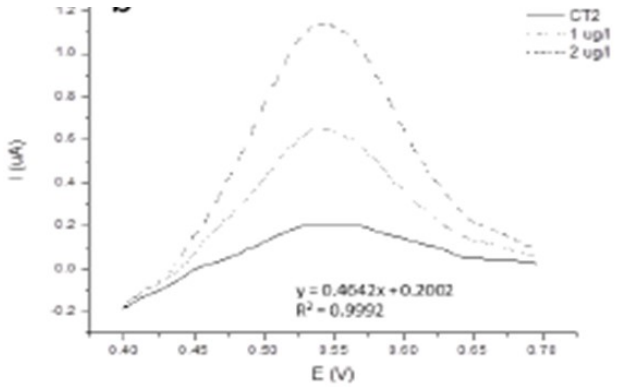


Figure 2. Voltammogram obtained from the analysis of a sample as such and after two standard additions of 2 µg/L of analyte.

Furthermore, a study had been carried out on the effect of the presence of cations and anions present simultaneously in solution with mercury, from which the selectivity of the technique had been highlighted.

The method can be used both for the determination of total mercury, and of Hg_{IN} and MeHg if present individually within the solution under analysis. In fact, all the chemical species of mercury respond in the same way to the technique, i.e. mercury (regardless of the form in which it is present in solution) is reduced to Hg^0 and forms and amalgam with the gold of which the electrode surface is constituted; consequently, following the oxidation in the stripping phase, the same reaction $Hg^0 \rightarrow Hg^{2+} + 2e^-$ always occurs, and gives rise to an oxidation peak at the same potential (Figure 3). It is therefore not possible to obtain distinct peaks for each of the two species under consideration.

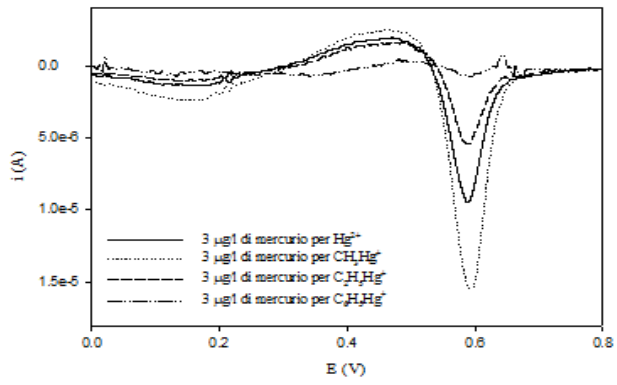


Figure 3. Voltammogram obtained for solutions containing 3 µg/L of Hg_{IN}, MeHg, ethylmercury and phenylmercury.

Problem solved

The project idea is to develop a system that, compared to existing ones, is

- faster,

- simpler,
- automatable
- which does not require the use of organic solvents,
- which does not require the use of gas,
- which does not require the use of microwaves,
- which works at room temperature,
- with lower purchase and management costs

in such a way as to allow to quantify both Hg_{IN} and MeHg in fish samples even in small laboratories (possibly set up in port areas, fish farms, distribution or processing companies) or in mobile stations set up on vehicles or boats dedicated to field analysis.

This would allow to:

- increase the number of checks on products;
- better understand the distribution of the two mercury forms along the food chain and in the environment;
- reduce downtime (this is perishable goods) and the associated costs (a ship standing in a port following the seizure of the goods transported, even just for the time necessary for the outcome of any analysis, costs on average 10,000 euros a day).

The progress of the project

Choice of the method

The analytical technique chosen for the determination of Hg_{IN} and MeHg is anodic stripping voltammetry with the square-wave potential scan mode (SW-ASV) using an SGE as WE. The choice of using an electrochemical technique arises from its simplicity of execution and versatility, as it allows the determination of different inorganic and organic chemical species present at the level of traces and ultratraces, from the possibility of performing on-site analysis quickly, and from the significant reduction in costs for the purchase and management of the instrumentation.

The choice to conduct the analyzes using an SGE derives from the results obtained in previous studies: it had allowed us to obtain excellent results in the determination of mercury thanks to its selectivity towards this metal, ensuring a more than sufficient sensitivity (the quantification limit determined was lower than the threshold limits set by law) and good repeatability. Moreover, being a solid electrode, it is easy to handle, it does not need further modifications that would require additional reagents and specialized personnel.

Samples analyzed

The method had previously been tested on a certified fish sample, namely ERM 464 (European Reference Material - Tuna Fish, certified by the Institute for Reference Materials

and Measurements of the European Commission), in order to evaluate its accuracy. It was very good since the recovery obtained was higher than 96% of the certified value for each considered sample.

Subsequently, the analysis of fish samples commonly found and widely consumed (canned tuna, swordfish, anglerfish, dogfish fillets, mussels ...) was carried out. In addition, we analyzed samples of cormorant liver, penguin feathers and cat food, provided by the Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta in order to assess whether the investigated was suitable. to monitor the phenomenon of biomagnification, i.e. the increase in mercury concentration along the food chain. Moreover, the feathers of seabirds could constitute an interesting type of sample to carry out environmental monitoring, as they are easily available and harmless for the animal.

Some samples were analyzed fresh, while others were first lyophilized and subsequently analyzed, in order to demonstrate the applicability of the technique we proposed to samples in different forms.

These samples at unknown concentration of Hg_{IN} and MeHg were also analyzed in parallel using DMA (official method used by the Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta which made the instrument available to us since it was a partner in the project, indicated in point 10 of this document, within which the experimental tests were conducted) and the concentrations found with this method were used as a "true" reference value.

The proposed method involves:

The use of disposable cartridges developed by us

An aliquot of commercial resin Amberlite XAD-1180 (a polymeric adsorbent). It is subsequently washed with ultrapure water (HPW), hydrochloric acid (10% v/v) and ethanol in order to remove inorganic impurities and any monomeric residues; afterwards it is left to dry at room temperature. Finally, it is functionalized with a commercial ionic liquid (IL), trihexyl(tetradecyl)phosphonium chloride (CYPHOS 101), with ratio resin: IL = 2: 1 in 5 mL of ethanol for 6 h. The suspension is finally filtered and the solid phase obtained (cyphos-modified XAD, CYXAD) is dried for 1 h in an oven at 60 °C, then stored at room temperature.

The CYXAD so prepared is stable and can be stored for long periods, making it possible to prepare batches of packaged cartridges ready for use.

The cartridges can then be inserted into the central part of a column support having a suitable housing, at the ends of which there are an inlet and an outlet tube to allow the passage of the sample solutions (Figure 4).

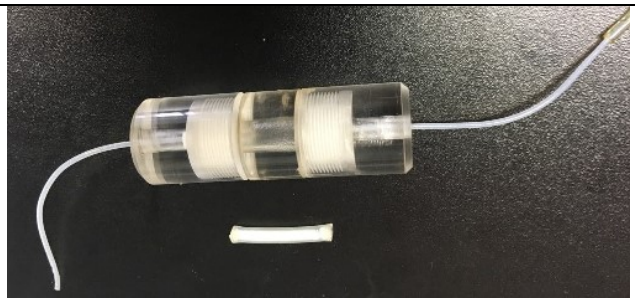


Figure 4. Cartridge and cartridge support (column)

Sample pretreatment

Aliquots of each sample are simultaneously subjected to two different pretreatment procedures.

1. For the determination of total mercury

- 0.5 g of lyophilized sample or 1 g of fresh fish are placed in contact with 3 mL of HNO_3 and 3 mL of H_2O_2 in a test tube and heated for 20 min at 60-70 °C. The heating is obtained by immersion in "bain-marie" in a portable food warmer (it can be recharged by connecting it to a fixed power socket or a field battery). The suspension is then filtered and the supernatant is diluted to 15 mL with HPW.

2. For the determination of Hg_{IN}

- 0.5 g of lyophilized sample or 1 g of fresh sample are placed in contact with 8 mL of 12 M HCl in a test tube and heated for 20 min at 60-70 ° C as described for "total mercury". The solution is then diluted 1: 3 with HPW to obtain a concentration of chloride ions of about 4 M, at which maximum resin efficiency is achieved. The solution is passed through a column containing the cartridge packed with the CYXAD solid phase: the latter holds Hg_{IN} quantitatively, while MeHg is eluted. The eluate containing MeHg is discarded because it has a too high Cl^-content that would damage the surface of the SGE.

Recovery of Hg_{IN} immobilized on the CYXAD takes place by elution with 5 mL of 6 M HNO_3.

Analysis by ASV.

An aliquot of each of the two solutions obtained in the pretreatments described above is transferred into a measuring cell and mercury is determined by SW-ASV with the SGE. The analysis can be performed both in the laboratory and on site with the same set of electrodes (WE: SGE, RE: Ag/AgCl/KCl), AE: platinum). Figure 5 and Figure 6 show, by way of example and not exhaustively, a benchtop and a portable instrument respectively.

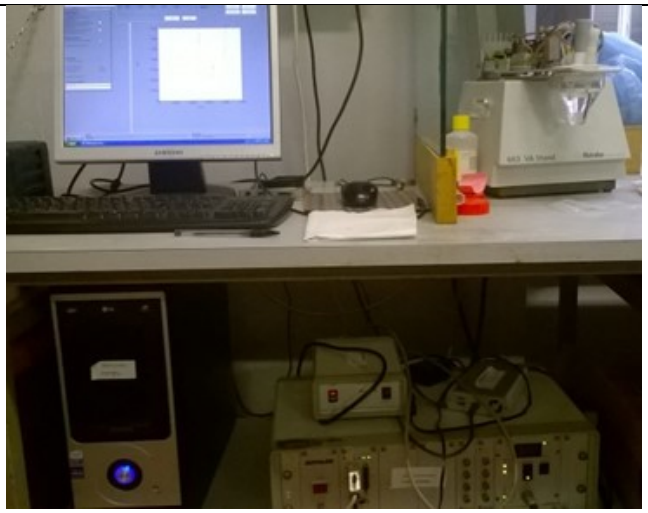


Figure 5. Benchtop voltammetric analyzer.

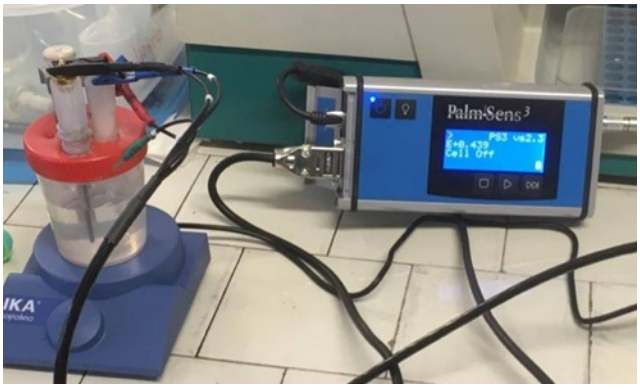


Figure 6. Portable voltammetric analyzer.

The quantification of mercury concentrations in the two solutions is carried out using the standard addition method, by adding aliquots of Hg^{2+} at known concentration into the electrolytic cell. The contents of total mercury and Hg_{IN} are thus determined. The concentration of MeHg is obtained by the difference between these two values.

The procedure is simple, easy to carry out and quick to implement. It does not involve the use of gases or organic solvents with a high environmental impact (used in the consolidated techniques for the determination of MeHg).

Results obtained in comparison with DMA

Tables 1 and 2 report the results obtained by SGE-ASV and DMA in terms of concentration of total mercury and MeHg in some of the samples analyzed.

Table 1. Total mercury concentrations (mg/kg) obtained by SGE-ASV and DMA.

Sample	Sample form	$[Hg_{TOT}]_{DMA}$ (mg/kg)	$[Hg_{TOT}]_{SGE}$ (mg/kg)
ERM-CE 464	Freeze-dried	5.00 ± 0.014	4.89 ± 0.56
ISPRA T22	Freeze-dried	4.21 ± 0.06	4.23 ± 0.22
Fillet of dogfish	Freeze-dried	5.40 ± 0.01	5.16 ± 0.01
Slice of tuna fish	Freeze-dried	2.11 ± 0.04	2.40 ± 0.08
Food for cat	Freeze-dried	0.27 ± 0.01	0.22 ± 0.02
Canned tuna	Freeze-dried	1.37 ± 0.04	1.21 ± 0.04
Cormorant's liver	Freeze-dried	4.16 ± 0.03	4.39 ± 0.14
Slice of swordfish	Fresh	2.42 ± 0.04	2.56 ± 0.29
Slice of emery fish	Fresh	2.90 ± 0.05	2.98 ± 0.21
Marlin Blue	Fresh	2.06 ± 0.03	2.07 ± 0.12

Table 2. Concentrations of MeHg (mg/kg) obtained by SGE-ASV and DMA.

Sample	Sample form	$[MeHg]_{DMA}$ (mg/kg)	$[MeHg]_{SGE}$ (mg/kg)
ERM-CE 464	Freeze-dried	4.01 ± 0.04	4.65 ± 0.02
ISPRA T22	Freeze-dried	4.11± 0.05	4.03± 0.04
Fillet of dogfish	Freeze-dried	4.70 ± 0.05	4.00 ± 0.05
Slice of tuna fish	Freeze-dried	1.82 ± 0.05	2.04 ± 0.01
Food for cat	Freeze-dried	0.25 ± 0.01	0.17 ± 0.01
Canned tuna	Freeze-dried	1.14 ± 0.01	1.12 ± 0.02
Cormorant's liver *	Freeze-dried	1.66 ± 0.02	3.57 ± 0.03
Slice of swordfish	Fresh	2.37 ± 0.03	2.52 ± 0.01
Slice of emery fish	Fresh	2.75 ± 0.04	2.35 ± 0.01
Marlin Blue*	Fresh	0.29 ± 0.01	1.95± 0.01

**Samples with high fat content*

Regarding the Hg_{TOT} content, the results obtained with our method have proved to be not significantly different from those obtained with the official method regarding the total mercury content. Plotting the results obtained with the two techniques we obtain a correlation coefficient (R^2) equal to 0.995.

For methylmercury an $R^2 = 0.996$ was obtained, not taking into account the "cormorant liver" and "marlin blue" samples. For these samples it seems, actually, that the procedure of extraction of MeHg performed for the subsequent determination with DMA does not allow a quantitative recovery. In fact, the content of this form is generally between 70 and 85% compared to total mercury. The percentages obtained for both samples were lower than 40% in the case of DMA, and greater than 80%. with the method proposed by us. The two samples in question showed a high percentage of fat that could have rendered the organic solvent extraction procedure used for the determination with DMA ineffective.

In conclusion, in Table 3 the main parameters of the method proposed by us are compared with those of the DMA.

Table 3. Analytical parameters (linear range, correlation coefficient, sensitivity, limit of detection (LOD), limit of quantification (LOQ): comparison between SGE-ASV and DMA.

Parameter	Matrix	DMA	SGE-ASV
Linear range	Blank	0.010-1.5 mg	0.2-100 mg/L
Correlation coefficient (R^2)	Blank	0.9996	0.9991
Sensitivity	Blank	3.2 Ass/mg[a]	1.71 μA/μg
LOD	Blank	0.001 μg	0.02 μg/L
LOQ	Blank	0.010 mg	0.2 mg/L
	Fish	0.037 mg[b]	0.5 mg/L[b]
		0.037 mg/kg[c]	0.3 mg/kg[c]

[a] Absorbance units
[b] Measured concentration
[c] Corresponding concentration in the fresh sample

As can be seen from the table, DMA has a greater sensitivity than the voltammetric technique, but the limit of quantification (in fish matrix) for the proposed method results to be 0.3 mg/kg, which is lower concentration than the legislative limit, so it is broadly sufficient to assess the healthiness of a fish product.

-

4. <u>INNOVATIVE TECHNICAL CHARACTERISTICS:</u>

1) Use of disposable cartridges selective for inorganic mercury

2) Possibility to develop kits for on-site analysis

The kit consists of:

- Test tubes previously filled with HNO_3/H_2O_2 mixture or with HCl.
- Vessel containing the washing solution (0.1 M $HClO_4$, 1.5 mM NaCl and 0.5 mM EDTA-Na_2) ready for use.
- Squeeze bottle containing HPW.
- Vessel containing Hg^{2+} standard solution.
- Vessel containing 60 mM NaCl.
- Scale to weigh aliquots of fresh or freeze-dried fish to be inserted into each tube.
- Food warmer with portable battery.
- Syringes to withdraw the sample solution at the end of the extraction and to transfer an aliquot into the measuring cell.
- Syringe filters to filter the aliquot of sample solution to be transferred into the measuring cell,
- Cartridges containing the CYXAD phase inserted in the column equipped with a disposable inlet filter.
- Motor-driven peristaltic pump or manual pump to favour the passage of solutions in the column.
- Portable cell with perforated cap for housing the electrodes.
- Micropipettes and tips for transferring the sample solution and adding known concentrations of mercury for calibration by standard additions
- Electrodes: SGE; RE: Ag/AgCl/KCl; AE: Pt.
- Portable potentiostat.
- Laptop computer.
- Portable battery.
- Container for the collection of wastes, consisting of sample solutions after analysis. The wastes can be transported to a laboratory to be properly disposed of, or delivered to specialized companies for disposal.

Figure 7 shows the kit used for on-site analyses.

Figure 7. Kit for on-site analyses.

3) Possibility to develop an automated instrument for speciation analysis in conventional or extemporaneous laboratories, since it only needs a power outlet.

- *Automated laboratory instrument*

The instrument will consist of:

- Thermostated autosampler with different positions: the samples are inserted into a carousel inside test tubes that are pierced by the tip of the autosampler. In odd positions, fresh or lyophilized samples are inserted in contact with the appropriate reagents (HNO_3 / H_2O_2 for Hg_{TOT} and HCl for Hg_{IN}); aliquots of washing solution (0.1 M $HClO_4$, 1.5 mM NaCl and 0.5 mM EDTA-Na_2) are added to the even positions. The tubes are thermostated at 70 ° C.
- Sample solution sampling system using a peristaltic pump.
  In the case of total mercury determination, an aliquot of the sample solution is directly driven into the measuring cell.
  In the case of the determination of inorganic mercury, an aliquot of the sample solution is driven into a disposable column (consisting of a prefilter and a cartridge containing the resin modified with the ionic liquid); inorganic mercury will be retained in the column in this phase, while methylmercury together with the hydrochloric acid solution will be transferred into the waste; then the retained inorganic mercury will be recovered by elution with nitric acid (contained in a vessel connected to an automatic dispenser) and transferred to the measuring cell.
- Measurement cell containing WE: SGE; RE: Ag/AgCl/KCl; AE: Pt.
- Vessel containing a solution of NaCl (supporting electrolyte) and metering pump to drive it into the cell together with the sample solution.
- Vessel containing a mercury standard solution and metering pump with which two standard additions will be performed directly into the cell.
- Suction system of the solution contained in the measuring cell which is sent to a waste after analysis.

The analysis involves the following stages:

- introduction of the samples and of the necessary reagents into the autosampler tubes. The samples for the determination of Hg_{TOT} and Hg_{IN} are treated directly in the tubes, thanks to the thermostatation;
- passage of an aliquot of washing solution through the lines and the cell;
- introduction of the sample solution and the supporting electrolyte (for the determination of Hg_{TOT}) or of the eluate exiting the column (for the determination of Hg_{IN}) into the cell;
- execution of the voltammetric analysis with the parameters previously set by the computer and recording of the voltammogram relative to the sample;
- addition of an aliquot of standard mercury solution to the cell, execution of the voltammetric analysis and recording of the voltammogram relative to the sample fortified with mercury. This stage is repeated twice;
- removal of the sample solution from the cell and transport into the waste;
- passage of an aliquot of washing solution through the lines and the cell;
- measurement of the peak height related to mercury and calculation of the concentrations of Hg_{TOT}, Hg_{IN} and (by difference) MeHg.

Figure 8 shows a scheme of the instrumentation required for laboratory analysis.

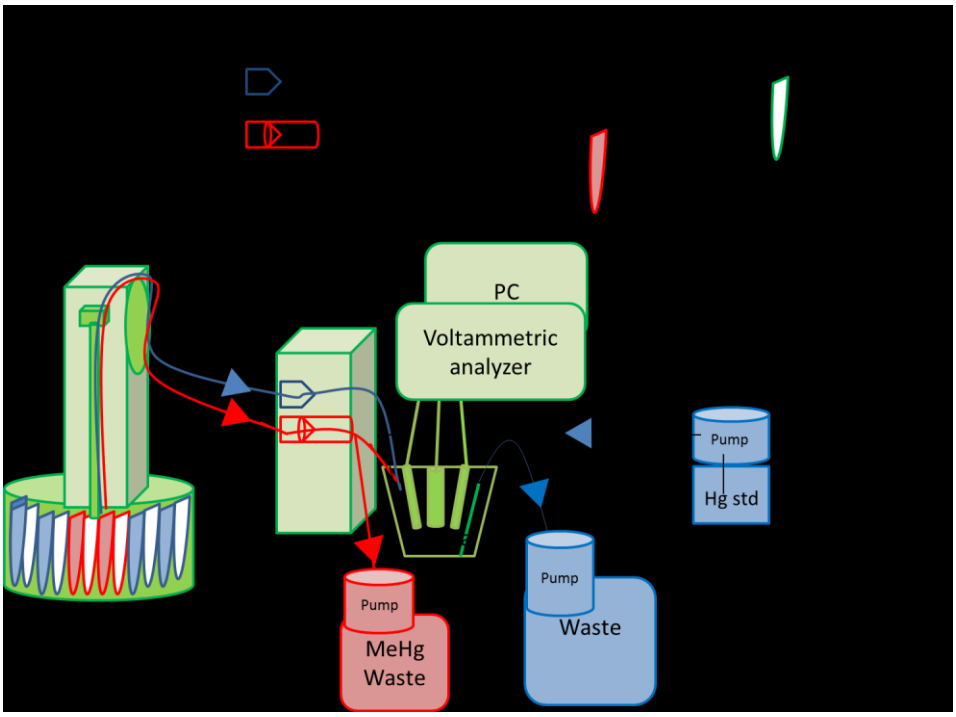


Figure 8. Scheme of the automated instrument for laboratory analyses

5. MAIN AREAS OF APPLICATION:

- Monitoring of fish products for the protection of consumer health;
- Screening analysis with portable kit directly on site;
- Environmental monitoring (evaluation of the distribution of different forms of mercury in waters, sediments, fish and seabirds).

6. STATO DELL'ARTE (eventuali brevetti e pubblicazioni noti nel campo dell'invenzione):

Nessuna ……..

……..

……..

……..

……..

7. PUBLICATIONS (IF ANY) CARRIED OUT BY THE PROPOSER/PROPOSERS IN THE FIELD OF THE INVENTION:

The proponents of the invention have been working for years in the field of the development of new analytical methods for the determination of trace metals and their application to the analysis of food and environmental matrices of various kinds (drinking water, fish, plants, food supplements, natural waters, sediments, soils, atmospheric particulate matter...). Specifically, the field of mercury determination has been extensively investigated using stripping voltammetry. This technique is well established but can be improved both from the point of view of analytical performance and from the perspective of developing techniques for analysis at low cost, low environmental impact and simple execution.

Giacomino A., Ruo Redda A., Squadrone S., Rizzi M., Abete M. C., La Gioia C., Toniolo R., Abollino O., Malandrino M., *Anodic stripping voltammetry with gold electrodes as an alternative method for the routine determination of mercury in fish. Comparison with spectroscopic approaches*. Food Chemistry, 221, 2017, 737.

Giacomino A., Schirinzi G., Pandi A., Malandrino M., Toniolo R., Abollino O., *New Easy Method for the Monitoring of Hg Concentration in Fish, Using a Nanostructured Gold Electrode,* Journal of Environmental Science and Engineering, 4, 2015, 378.

Giacomino A., Abollino O., Malandrino M., and Mentasti E., *Voltammetric determination of methylmercury and inorganic mercury with an home made gold nanoparticle electrode,*

Talanta, 85, 2008, 266.

Giacomino A., Abollino O., Malandrino M., Mentasti, E., *Parameters affecting the determination of mercury by anodic stripping voltammetry using a gold electrode.* Talanta, 75, 2008, 266.

Abollino O., Giacomino A., Ginepro M., Malandrino M., Zelano I., *Mercury (II) trace detection by a gold nanoparticle-modified glassy carbon electrode using square-wave anodic stripping voltammetry including a chloride desorption step.* Electroanalysis, 24, 2012, 727.

Abollino O., Giacomino A., Malandrino M., Piscionieri G. Mentasti E., *Determination of mercury by anodic stripping voltammetry with a Gold Nanoparticle–modified Glassy Carbon Electrode,* Electroanalysis 20, 2008, 75-83.

……..

8. EVENTUALI CONTATTI IN CORSO (ed eventuali accordi di riservatezza stipulati in merito):

There are ongoing contacts not yet materialized both with companies producing analytical instruments, and with associations operating in the fishing industry and the processing of fish products, interested in financing the research of a method of analysis allowsing a rapid identification of any critical issues along the chain of collection and processing of fish products.

9. SOGGETTI CONTITOLARI E/O CO-INVENTORI (segnalando la relativa posizione giuridica rispetto all'Università di Torino e la percentuale del contributo prestato):

Dott.ssa Agnese Giacomino, . Ricercatore a tempo indeterminato, Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino; 45 %.

Dott. Andrea Ruo Redda, Borsista Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino; 35 %.

Prof.ssa Ornella Abollino, Professore Associato, Dipartimento di Chimica, Università di Torino; 10 %.

Prof.ssa Mery Malandrino, Professore Associato, Dipartimento di Chimica, Università di Torino; 10 %.

10. LINEA DI RICERCA DA CUI DERIVA L'INVENZIONE (segnalando anche le fonti di finanziamento):

Project financed by Fondazione CRT in the framework of 2016 ordinary requests.

PI: Dr. Agnese Giacomino, Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino.

Title: « Development and application of electrochemical sensors for the determination of mercury and methylmercury in environmental and food matrices ».

Funding: € 35,000.

I SOTTOSCRITTI DICHIARANO CHE LA PRESENTE RICHIESTA NON CREA CONFLITTO CON ALTRE PERSONE FISICHE E/O ENTI CHE POSSANO AVANZARE DIRITTI SULL'INVENZIONE STESSA.

DATA 25/09/2018 FIRMA DELL'INVENTORE/DEGLI INVENTORI

Giacomino Agnese (45%) 

Ruo Redda Andrea (35%)_  _

Abollino Ornella (10%) 

Malandrino Mery (10%) _ 

TRATTAMENTO DATI:

si autorizza il trattamento dei dati personali (di cui al punto 1) ex L. 675/96 nell'ambito delle finalità di cui alla presente richiesta

DATA 25/09/2018 FIRMA DELL'INVENTORE/DEGLI INVENTORI

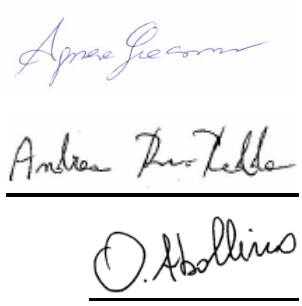
DOCUMENTO RISERVATO

Allegato b)

IMPEGNO ALLA RISERVATEZZA

I SOTTOSCRITTI DICHIARANO DI AVER MANTENUTO E SI IMPEGNANO A MANTENERE ASSOLUTA RISERVATEZZA IN MERITO ALL'OGGETTO DELL'INVENZIONE PER LA QUALE SI CHIEDE LA TUTELA BREVETTUALE, SALVO LA POSSIBILITA' DI PUBBLICARE/DIVULGARE I RELATIVI DATI IN UN MOMENTO SUCCESSIVO ALLA DATA DI DEPOSITO DELLA DOMANDA DI BREVETTO

Data 25/09/2018 **Firma dell'inventore/degli inventori**



Data **Firma dei componenti della Commissione Tecnica Brevetti**

……

……

……

……

……

……

……

……

Data **Firma del responsabile del competente ufficio dell'Amministrazione Centrale**

……