SLOVENSKI STANDARD
SIST ISO 15089:2010

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Kakovost vode - Smernice za selektivno imunoanalizo za določevanje aktivnih snovi v sredstvih za zaščito rastlin in v pesticidih

Water quality - Guidelines for selective immunoassays for the determination of plant treatment and pesticide agents

Qualité de l'eau - Lignes directrices relatives aux dosages immunologiques sélectifs pour la détermination des agents de traitement des plantes et des pesticides

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Water quality — Guidelines for selective immunoassays for the determination of plant treatment and pesticide agents

Qualité de l'eau — Lignes directrices relatives aux dosages immunologiques sélectifs pour la détermination des agents de traitement des plantes et des pesticides

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 15089 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical, biochemical methods*.

Annexes A and B of this International Standard are for information only.

Water quality — Guidelines for selective immunoassays for the determination of plant treatment and pesticide agents

1 Scope

This International Standard specifies a guide for the selective quantitative analysis by immunoassays of environmental chemicals such as pesticides (including insecticides) or their metabolites in drinking, ground and surface water.

The application range of the procedure for the analysis of pesticides in drinking water applies to mass concentrations $\geqslant$ 0,05 µg/l. Therefore, the determination limit should be in this case $\leqslant$ 0,05 µg/l.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes.*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples.*

ISO/TR 13530:1997, *Water quality — Guide to analytical quality control for water analysis.*

3 Terms and definitions

For the purposes of this International Standard, the following terms and definitions apply.

3.1
affinity
strength of binding of antibody to analyte

NOTE The strength is defined by the equilibrium constant (K) of the reaction Ab + H = AbH, where Ab = antibody combining site and H = hapten; K is given by the mass action equation $K = c_{\mathrm{AbH}}/(c_{\mathrm{Ab}} \times c_{\mathrm{H}})$.

3.2
analyte
substance to be determined

3.3
antibodies
serum proteins produced in vertebrates in response to immunization and which selectively bind the antigen or hapten, respectively

NOTE 1 Monoclonal antibodies (mAb) are uniform populations of antibodies which are produced from a single cell clone of hybridoma cells.

NOTE 2 Polyclonal antibodies (pAb) are a mixed population of antibodies which are produced by several clones of plasma cells.

NOTE 3 Recombinant antibodies (rAb) are produced using recombinant techniques established in gene technology.

3.4
antibody conjugate
antibody covalently linked to a label such as an enzyme or a fluorochrome

3.5
antigen
substance that stimulates the production of antibodies and reacts with them

3.6
antiserum
immune serum obtained from the blood of immunized vertebrates after removal of cellular components and coagulation factors

NOTE It usually contains a number of different antibodies which can exhibit different affinities to the antigen/hapten.

3.7
coating conjugate
macromolecule bound to the hapten (also known as a hapten-carrier conjugate), which is immobilized to a solid phase

NOTE It is used to bind those antibody binding sites which are not occupied by the analyte.

3.8
competitive immunoassay
test which detects the proportion of antibody binding sites which have been occupied by the sample analyte

NOTE This is achieved by adding a tracer which binds to the unoccupied antibody binding sites and produces the measuring signal after a further reaction.

3.9
cross-reactivity
extent, to which an antibody or an antiserum reacts with a substance which structurally differs from the analyte

NOTE 1 The cross-reactivity of an antibody or an antiserum with this substance is determined by comparing the calibration curves. The reference curve obtained with the analyte is used as reference quantity (= 100 % cross-reactivity). The cross-reactivity is usually determined at the IC_{50}. The selectivity of an antibody or an antiserum, respectively, is inversely related to the cross-reactivities. An antibody or an antiserum, respectively, can display different affinities to different substances. With a given substance, the cross-reactivity of an antiserum can also vary within the measuring range. Usually the structure of the immunogen essentially determines the selectivity (the so-called specificity) of an antiserum. If cross-reactivities are due to a mixed population of antibodies as often occurs in an antiserum, the undesired antibodies may be removed by cross-absorption.

NOTE 2 All compounds (present in relevant concentrations) that exhibit cross-reactivity will create false positive results.

3.10
enzyme immunoassay
EIA
immunochemical analysis which detects the occupancy of antibody binding sites by the analyte with the aid of a tracer (an enzyme-labelled hapten or an enzyme-labelled antibody) and consequently can be used to detect the analyte concentration in the medium

NOTE The detection procedure is based on the measurement of the enzyme activity of the tracer by means of substrate conversion.

3.11

enzyme substrate

substance which is converted by the enzyme into a product that can be detected by a measuring device

3.12

excess standard

analyte concentration which, once exceeded, produces no further decrease in the signal measured in the immunoassay

3.13

fluorescence immunoassay

immunochemical detection procedure which is performed either as an immunoassay with fluorescent substrates or products, respectively, or as an immunoassay with fluorescence-labelled tracers or antibodies, respectively

3.14

hapten

substance which, because of its small molecular size, does not evoke the production of antibodies unless it is covalently bound to an immunogen

NOTE Pesticides are examples of haptens.

3.15

heterogeneous immunoassay

test which requires a separation of solid phase-bound and unbound tracers in order to detect the occupancy of antibody binding sites by the analyte and thus the analyte concentration in the sample

3.16

immunoassay

quantitative assay which is based on the selective analyte/antibody binding and uses tracers for the detection of the free or occupied antibody binding sites, respectively

3.17

immunogen

substance which triggers an immune response after injection into a vertebrate

3.18

inhibition concentration

IC

analyte concentration which reduces the measuring signal of the zero standard (= 100 %), in the case of IC_{50} to 50 % of the zero standard

3.19

luminescence immunoassay

LIA

immunochemical assay which is either performed as an immunoassay detecting luminescent substrate or product, respectively, or luminescence-labelled tracer

3.20

solid phase immunoassay

heterogeneous immunoassay which uses (depending on the test type) antibodies or coating conjugates, immobilized to a solid phase

NOTE Usually, both test types are known as ELISA (enzyme-linked immunosorbent assay) when enzyme-labelled tracers are used.

3.21

tracer

labelled hapten (or antigen) or labelled antibody which, in the case of competitive immunoassays, is used to detect the percentage of antibody binding sites not being occupied by the analyte

3.22
zero standard
analyte-free standard (method blank) which is used for calibration

4 Interferences

Interferences are caused by improper sampling, for instance due to the choice of equipment or materials which adsorb or liberate the substances to be analysed. Assay conditions, for instance pH or sample components such as metal ions, humic acids, salinity and solvents influencing the test components (for instance matrix effects), can interfere with the analysis. The influence of matrix effects may be assessed by spiking samples with known amounts of the analyte.

5 Principle

Immunoassays are methods which use antibodies produced against defined analytes or analyte groups as biochemical sensors for the quantification of analyte concentrations. These assays are particularly useful as screening assays. All immunoassays for the detection of haptens are based on the principle of the competitive immunoassay. Assays for pesticides have been reported (see references [1] to [4] in the bibliography). A typical procedure, as an example of an EIA, is described below.

The solid phase variant requires either immobilized antibodies and dissolved hapten tracers (variant a, direct immunoassay) or immobilized coating conjugate and dissolved antibody tracers (variant b, indirect immunoassay) in constant ratios. The antibodies are applied in limiting amounts. Therefore components which are not bound to the coated solid phase can be removed by washing prior to the final detection. This also includes most of the interfering matrix effects. The more tracer molecules are bound, the lower is the analyte concentration in the sample.

The immobilization can be achieved by passive adsorption or by covalent binding to functional groups of the solid phase.

The calibration is performed with solutions of known analyte concentrations.

6 Reagents

6.1 General

Use reagents and water of high purity (for instance "for residue analysis"). Information on type and origin of antibodies or antisera, respectively, as well as the cross-reactivities shall be stated in the report. Information on storage and stability of the used reagents shall be requested from the supplier. The selectivity of the antibodies or antiserum, respectively, shall guarantee that sample concentrations deduced from a calibration curve do not deviate by more than ± 10 % from the actual analyte concentrations in these samples. If the specificities of the antibodies or antiserum, respectively, are low, the interfering analyte shall be removed from the samples by suitable chemical-physical procedures. Cross-reacting antibodies of an antiserum can be removed by cross-absorption.

6.2 Buffered washing solution, used for washing for instance phosphate buffered saline (PBS; pH 7,6) with a phosphate concentration of 80 mmol/l (prepared with NaH_2PO_4 and Na_2HPO_4) and a sodium chloride concentration of 85 g/l.

6.3 Acid, for instance sulfuric acid, $c(H_2SO_4) = 1$ mol/l, for adjusting the pH and stopping the enzyme reaction.

6.4 Base, for instance sodium hydroxide, $c(NaOH) = 1$ mol/l, for pH adjustment and for stopping the enzyme reaction.

7 Apparatus

7.1 Solid phase, consisting of plastics, glass or magnetic particles for use in heterogeneous immunoassays, for instance microwell plates, test tubes, beads, magnetic particles, or membranes.

7.2 Multipipettes, for instance variable pipettes 10 µl to 500 µl, fixed volume pipettes 10 µl, 100 µl, 200 µl, multichannel pipettes for instance 300 µl, and dispensers.

7.3 Magnetic rack, consisting of a rack with a magnetic base to be used during the washing step for immunoassays with antibodies bound to ferromagnetic particles.

8 Sampling and sample preparation

8.1 Sampling

Perform sampling in accordance with ISO 5667-1, ISO 5667-2 and ISO 5667-3. The immunoassay is generally performed with raw water samples. Sample pretreatment should be considered, if necessary. It is possible to concentrate samples by suitable chemical-physical procedures.

8.2 Coating of solid phases

Coated solid phases can be obtained commercially. Otherwise they should be prepared for enzyme immunoassays as follows:

a) by incubating diluted solutions of the antibody or antiserum, respectively, in a suitable buffer solution, for instance 50 mmol/l carbonate buffer (pH 9,6), prepared with Na_2CO_3 and $NaHCO_3$. This is sometimes referred to as direct ELISA;

b) coating the solid phases with a hapten-carrier conjugate before starting the assay. This is sometimes referred to as indirect ELISA.

Usually proteins are applied as carriers. After coating and a subsequent washing step, blocking of the unspecific binding sites can be necessary for some assays. Furthermore, it is necessary to prepare a standard calibration series of the respective pesticide, for instance in distilled water.

8.3 Enzyme tracer

The hapten-enzyme tracers (direct ELISA) or antibody-enzyme tracers (indirect ELISA) used for the enzyme immunoassay are either prepared shortly before use in a suitable dilution in buffer solution or they are provided by the supplier in a stabilized form ready for use. The enzyme substrate is prepared shortly before use by dissolving one or more components in a buffer solution or it is obtained in a stabilized form from the supplier.

8.4 Solutions

Prepare washing solutions for rinsing the solid phase (see 9.1.2) as well as suitable acids or bases for terminating the enzyme reaction (see 9.1.3) prior to the measurements.

8.5 Sample preparation

Prior to the assay, all water samples, reagents, solutions, equipment and coated solid phase are brought to a defined temperature. The enzyme reaction is carried out at constant temperature (± 0,5 °C) in the range between 20 °C and 37 °C. If the pH of the water samples is not in the range between 7,0 and 7,5 or another value is chosen as suitable for the assay, a pH adjustment (for instance with NaOH or H_2SO_4) should be carried out.

The resulting dilution of the samples has to be taken into account in the calculation.