CLAIRO CONFERENCE: LIVABLE AND CLIMATE RESILIENT EUROPEAN CITIES

How to strengthen resilience of greenery with biofertilizers

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22 March 2022

Fields of our interest

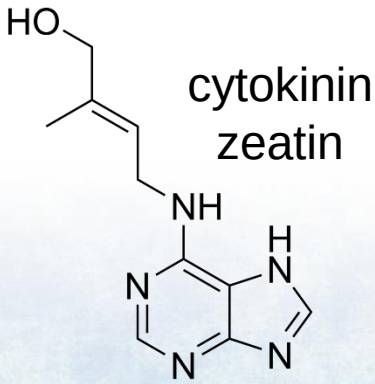
- isolation, identification and quantification of endogenous plant hormones, their biosynthesis, metabolism and cross-talk

- synthesis and structure-activity relationships, development of new plant hormone derivatives for agriculture and biotechnology

- cdk inhibitors, apoptosis, cytotoxicity

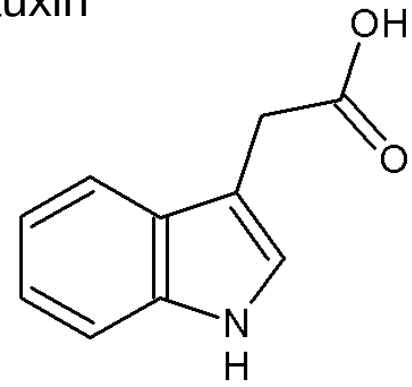
- imunoanalytical chemistry of plant hormones

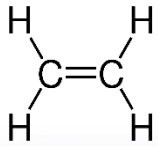
- phytohormones, signal molecules produced within the plant, play crucial role in the control of growth and development (formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit, shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruits, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death.



cytokinin
zeatin

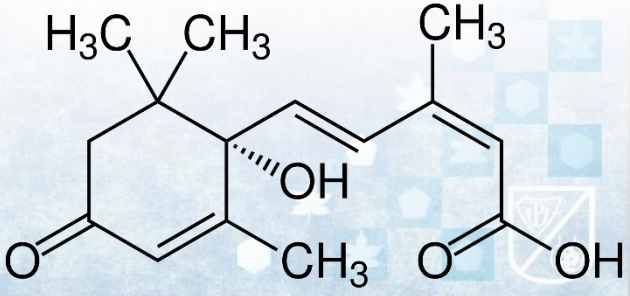
indolyl-3-acetic acid
auxin





ethylen

Abscisic acid



Plant Hormones

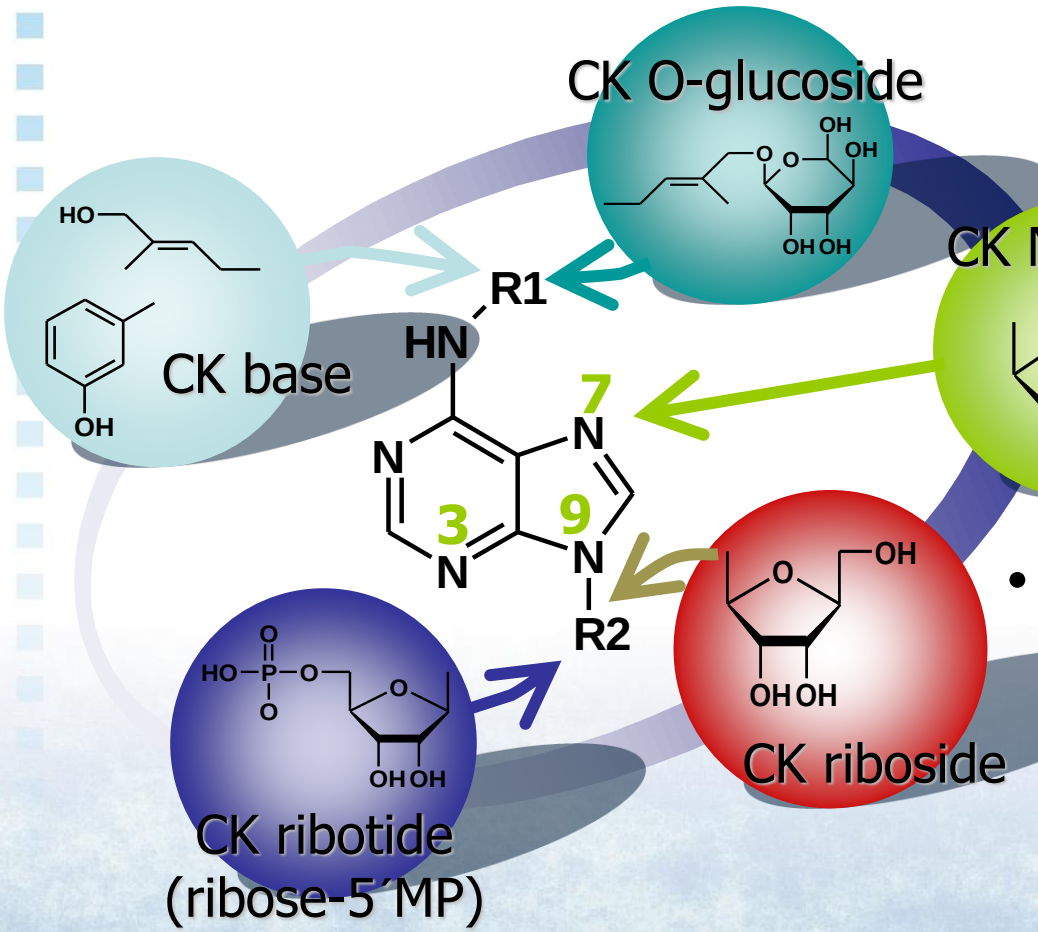
- low concentration in plants: 10^{-10} -10^{-15} *mol/g F.W.*
- very low levels of phytohormones is a major problem associated with their analysis

- Basic groups:
- Cytokinins: **0.01 – 100 pmol/g**
- Auxins: **0.1 – 100 pmol/g**
- Gibberellins: **0.1 – 10 pmol/g**
- Abscisic Acid: **0.1 – 1000 pmol/g**
- Ethylene: **1 – 100 pmol/g**
- Brassinosteroids: **0.001 – 10 pmol/g**
- Strigolactones

- Phytohormone-like compounds:
  - Jasmonates
  - Polyamines
  - Phenolic Acids
  - *others*

Cytokinins(CK)

- CKs regulate cell division in shoots and roots, growth of stems and roots and specific components of the cell cycle.



- CKs overproduction is implicated in plant tumour development.
- CKs delay leaf senescence.
- CKs promote chloroplast development and cell extension in leaves and cotyledons.

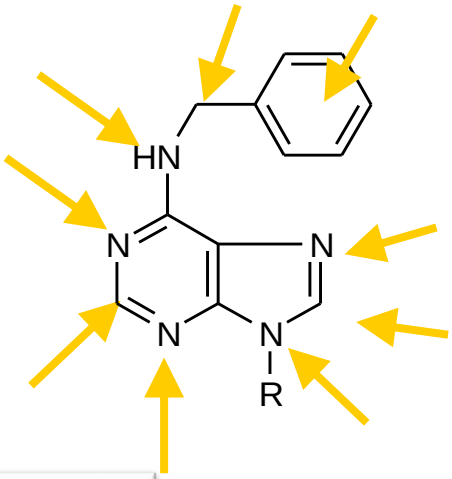
used methods

- HPLC-ELISA, HPLC-MS, UPLC-MS/MS
- polyclonal, monoclonal antibodies, immunoaffinity chromatography
- organic synthesis and compound characterization (MS, NMR, X-ray diffraction)
- plant and animal tissue cultures, cytokinin bioassays, AHK receptor assays, CKX assays
- $p34^{cdc2}$ inhibition, cytotoxicity and anti-inflamatory activity determination

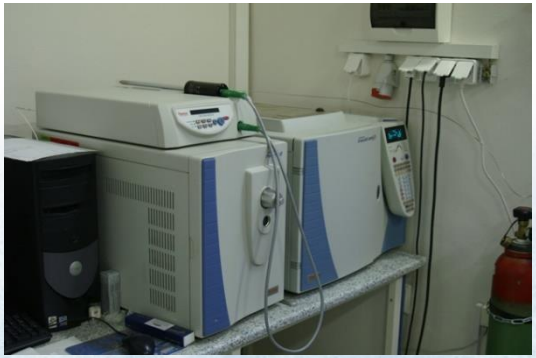
Increase the resistance of greenery against abiotic stress

- Palacký University in Olomouc
- Preparation of stimulatory compouds specifically for selected species and type of stress

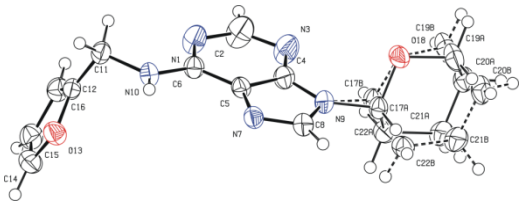
Design and preparation of new phytohormone derivatives:



- CEM Discover SP microwave reactor and X-cube flow reactor (reaction time in minutes)



Synthesis



Identity and purity characterization

Biological activity *in vitro /in vivo* plant models

Biological activity *in vitro /in vivo* animal models

Plant tissue cultures

Phenotyping

Field experiments

Patent protection

Biostimulants

Plant biostimulants contain substance(s) and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides.

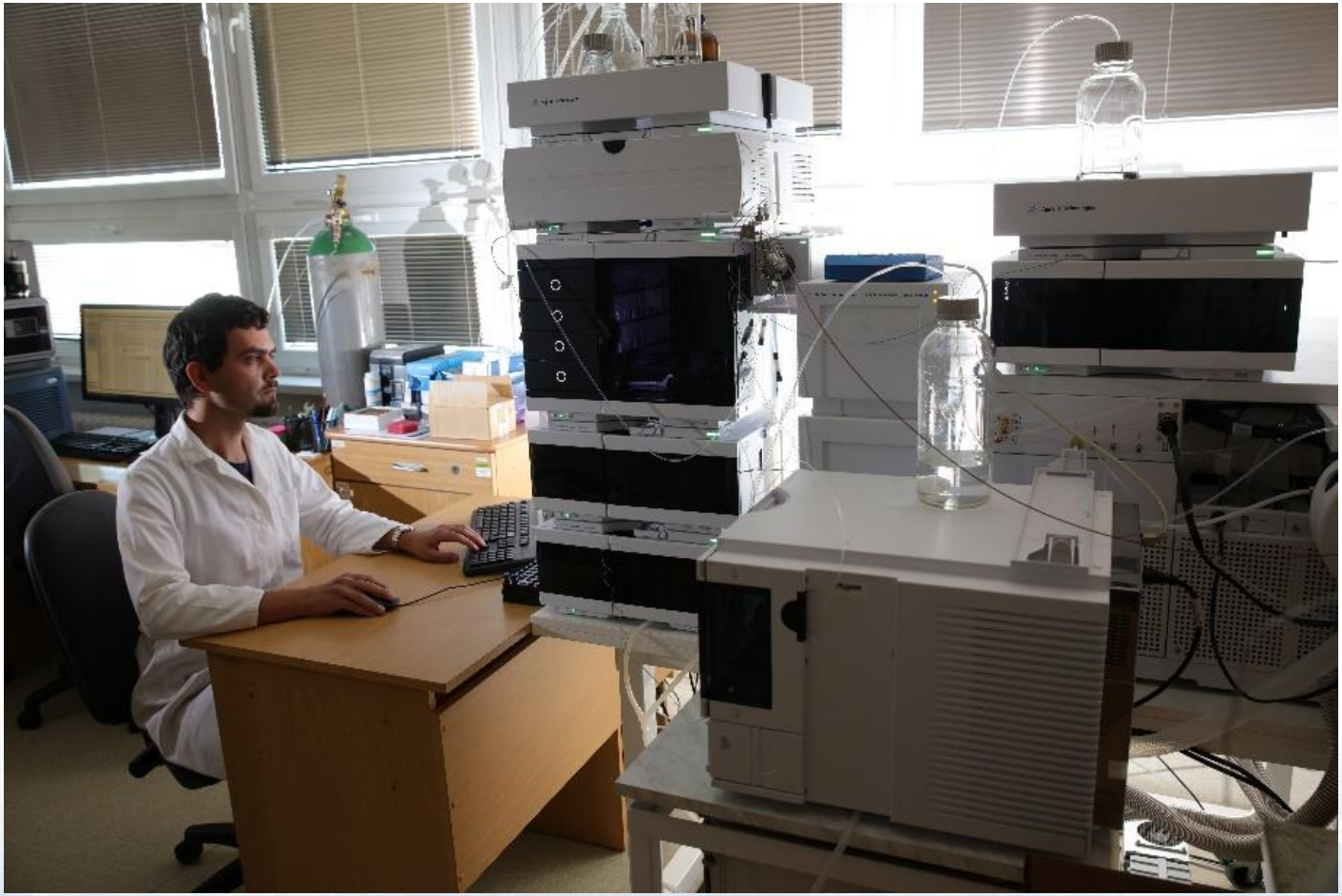
Plant growth promotion resulting from microbial inoculants is due to increased production of plant hormones. The non-microbial biostimulants are further classified as organic biostimulants [seaweed extracts, protein hydrolysates, humic substances, smoke–water (SW), vermicompost leachate, chitosan and plant extracts]. Many of them also contain significant levels of plant hormones.

They can be modern alternative to the clasic inorganic fertilizers, which can be overused.

- **Non-destructive monitoring of physiological state of plants** in polluted regions using small but precise instruments. **Chlorophyll Meter SPAD**-502DL enables very fast and non-destructive determination of relative chlorophyll content in leaves with no need of pigment extraction. We can measure also **fluorescence of chlorophyll *a* in leaves**, which reflects a wide range of processes in plant photosynthetic apparatus and is a **significant and fast indicator of plant stress**. **Fluorometer PEA** (Hansatech, UK) measures fast and non-destructive so-called **fast chlorophyll fluorescence induction** (O-J-I-P curve) enabling e.g. a computation of **maximal quantum efficiency of photosystem II** (PSII) photochemistry - F_V/F_M. **In stress conditions, F_V/F_M parameter decreases** because of impairment of PSII function. Therefore, F_V/F_M is the fluorescence parameter most frequently used for photosynthesis monitoring in stress conditions.

In addition, we can use a unique **portable gasometric system** **LI-6400** (LI-COR Biosciences, Inc., Lincoln, NE, USA) **enabling non-invasive simultaneous measurements of gas-exchange parameters** (rate of CO_2 assimilation, transpiration, stomatal conductance, CO_2 concentration in intercellular spaces) and **chlorophyll fluorescence parameters on a plant leaf**. Except mentioned F_V/F_M, LI-6400 is able to measure also so-called slow chlorophyll *a* fluorescence induction, which shows a decline (quenching) of measured chlorophyll fluorescence intensity in time. An enhancement of chlorophyll fluorescence quenching may indicate an acclimation of photosynthetic apparatus on deteriorative ambient conditions with intended generation of quenching centres that should protect functional photosynthetic reaction centres. Thus, LI-6400 provides complex information about a state of primary and secondary photosynthetic reactions included proportion of stomata closing/opening, **all those parameters are significant and fast indicators of stress/acclimation of plants**.

Plant Hormone Quantification – UHPLC-MS/MS



Phytohormone analysis

The concentration in plants: ***10 $^{-10}$ -10 $^{-15}$ mol/g F.W.***

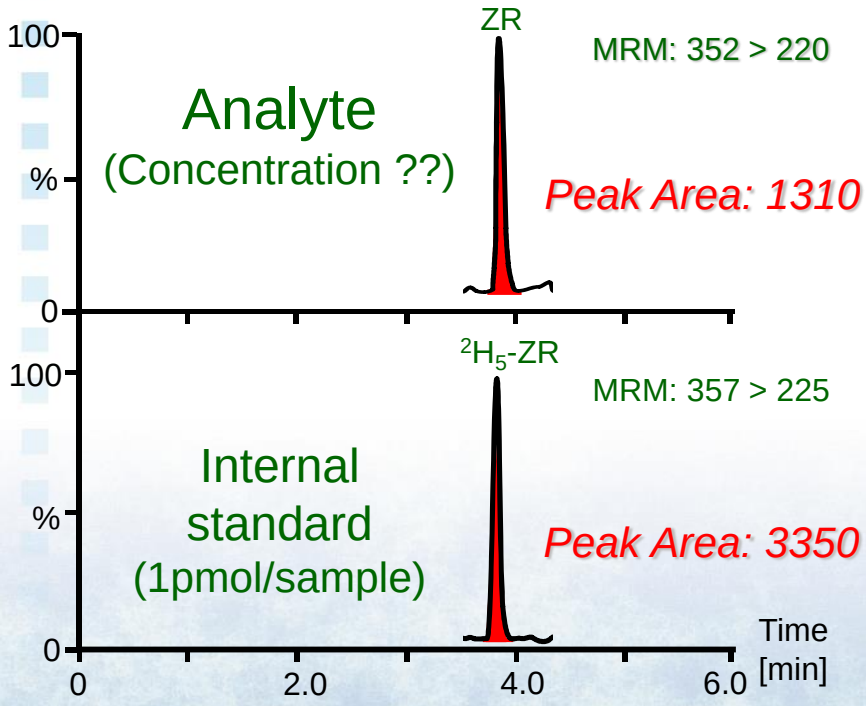
The method can be divided in two basic parts:

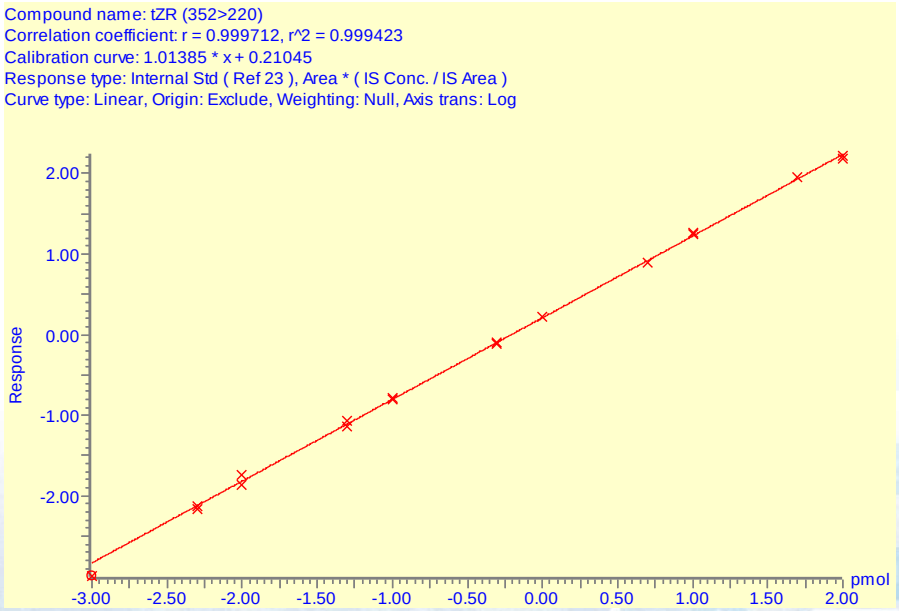
1) purification of plant samples
   - extraction of plant tissues and addition of internal labelled standards (^{2}H, ^{15}N, ^{13}C, ^{18}O)
   - pre-concentration of the extract using solid phase and ion exchange purification
   - isolation of phytohormone metabolites using immunoaffinity chromatography
2) quantification and identification by liquid chromatography/ mass spectrometry
   - a sensitive UHPLC/(ESI)-MS/MS method for quantitative analysis
   - an accurate identification based on exact mass determination by LC/(ESI)-QqTOF

Quantitation by isotope dilution analysis

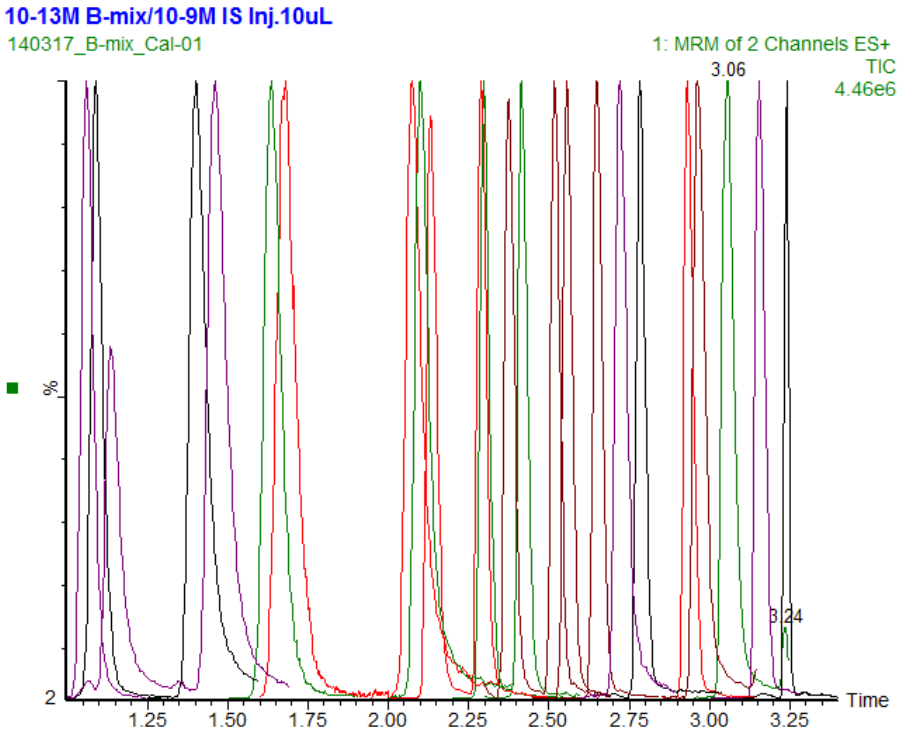
- addition of internal (stable isotope) standard during purification
- control of purification procedure and MS determination

$$\textit{Amoung of endogenous compound in sample} = \frac{\textit{Peak area of endogenous ion}}{\textit{Peak area of internal standard ion}} \times \textit{Amount of internal standard added}$$





Fast scanning LC-MS Method



(A) Methanol

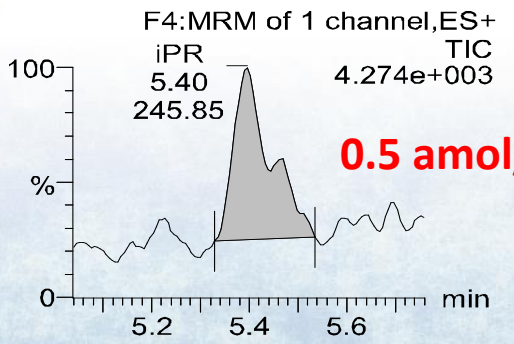
(B) 15 mM $HCOONH_4$, pH4

UHPLC: BEH C18 (2.1x50 mm, **1.7µm**)

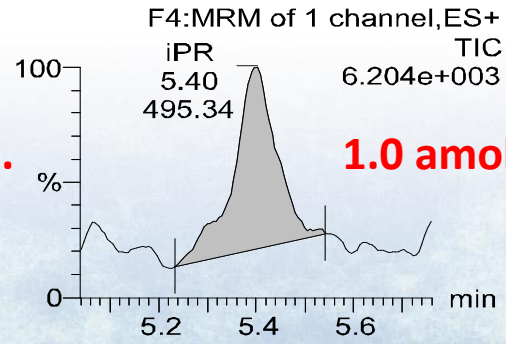
Flow rate: 1ml/min Temp: 50°C

- Detection limits close to **1 attomolar level**
- Retention time stability lower than **0.5% (RSD)**

Hypersensitivity of new Generation UHPLC-MS/MS (ACQUITY UPLC I-Class system / Xevo TQ-S MS, Waters)



0.5 amol/inj.



1.0 amol/inj.

Novák *(unpublished)*

Thank you for your attention.