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ECOLETTER

ACCELERATED STORAGE STABILITY OF CITOGROWER®

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INTRODUCTION

Change in content of active ingredient throughout time is an indicator of the stability of a particular product. The stability of agrochemical products is commonly evaluated by measuring periodically the concentration of an individual ingredient of the formulation while stored at high temperatures. The guidelines of Collaborative International Pesticide Analysis Council (CIPAC) establish that ageing of a particular product can be accelerated through storage at 54ºC during 14 days or at 45ºC during six weeks. In the case of thermo-sensible products, evaluation is done at lower temperatures but storage time should be extended accordingly. In any case, a positive result in this test means that the product will remain stable for at least two years or more under normal storage conditions (room temperature).

CITOGROWER® is a foliar fertilizer containing phosphorous, potassium, L-free amino acids and adenine-derived compounds with activity equivalent to cytokinins. Application of this product in flowering stage improves yield and quality of fruits at harvest, mainly by the action of phytohormonal compounds.

There are two types of cytokinins: adenine-type cytokinins represented by kinetin, zeatin and 6-benzylaminopurine, as well as phenylurea type cytokinins like diphenylurea or thidiazuron (TDZ).

Considering the importance of phytohormonal compounds into the CITOGROWER® formulation, in this study the stability of the product was assessed following the content of 6-bencialminopurine (6 BAP) in samples of the product stored at 45ºC and 54ºC during six and two weeks respectively. Variation lower than 15% in such content would indicate that the product is stable for at least two years (CIPAC, 1995)

MATERIALS AND METHODS

The study was done on 1 L sample of CITOGROWER®, batch number A2844D7, manufactured on 04/04/2007 (expirity date: 04/04/2009).

Storage at 54ºC:
Three 20mL sub-samples were taken from the original product and stored at 4ºC (time 0, reference) in refrigerator, at room temperature (min 15ºC max 25ºC) in lab shelf and at 54ºC±0.2ºC in an incubator (INCUDIGIT, JP SELECTA) during 14 days.

Storage at 45ºC:
Eight 20mL sub-samples were taken from the original product and labelled as T45-1, T45-2, T45-3, T45-4, T45-5 and T45-6, T45-A and T45-0. They were simultaneously stored at 45ºC±0.2ºC (samples T45 1-6) into an incubator (INCUDIGIT, JP SELECTA), at 4ºC (T45-0) in refrigerator and at room temperature (T45-A) in lab shelf. A sample was recovered from the incubator each seven days and stored at 4ºC until the end of storage period. The sample stored at room temperature was transferred to refrigerator (4ºC) along the last sample removed from incubator.

Chromatographic analysis:
At the end of each storage period (14 days at 54ºC or six weeks at 45ºC), the samples were simultaneously analysed by HPLC to determine the content of 6-benzylaminopurine.

Extraction:
Each sample was diluted 1/10 and 5mL from each dilution were transferred to a 50 mL volumetric flask. Then 40mL of an extractive mixture of methanol and HCl 0,1N (1:1) was added to each flask. The flasks were put in ultrasound bath for 10 minutes and were additionally agitated for 10 minutes. The volume was rise up with the extractive mixture and the content was filtered through a 0,45 μm membrane.

High Performance Liquid Chromatography:
A standard HPCL instrument adjusted to conditions described in Table 1 was used. Then samples of 5 μL from extracts were injected. The validation was done with dilutions from standard reference at concentrations of 1, 0.2 and 0.02 mg/mL. Each sample was analysed three times.

Column	Spherisorb ODS2 25 x 0.4 cm 5µm
Mobile phase:	Phosphate buffer NaH_2PO_4/Methanol (1:1)
Injection volume :	5 µL
Flow:	1mL/min
Detection	UV at λ =267 nm
Retention time:	16 min
Analysis time:	20 min

Table 1. *Chromatographic conditions*

RESULTADOS

The evolution of concentration of 6BAP in samples stored at 45ºC and 54ºC compared with samples stored at room temperature is showed in Figures 1 and 2 respectively.

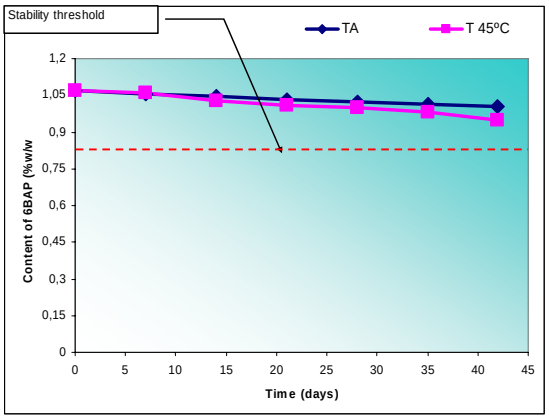

Figure 1. *Evolution of concentration of 6BAP in sample of CITOGROWER® stored at 45ºC*

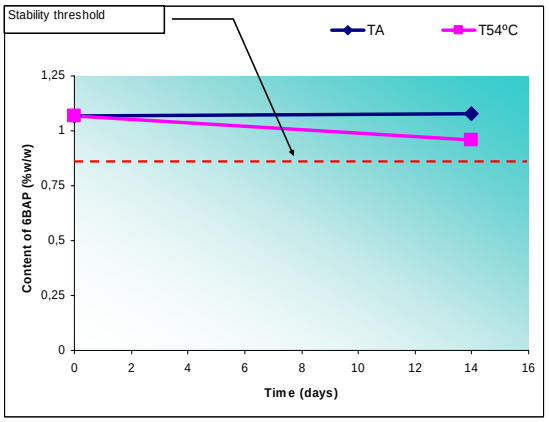

Figure 2. *Evolution of concentration of 6BAP in simple of CITOGROWER® stored at 54ºC*

The method was validated with a standard reference of 6BAP using the chromatographic conditions described (Table 1). The parameters analysed were work interval, detection limit, linearity and accuracy. It was found that the minimum detectable concentration of 6BAP in the analytical method used was 0.0018 g/L, linearity 0.9998 and accuracy 0.0046 (Figure 3).

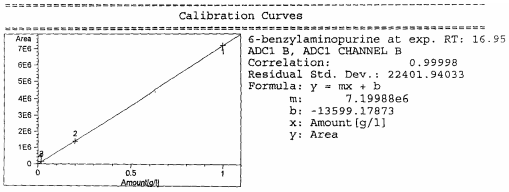

Figure 3. *Results of validation of method of determination of 6BAP in samples of CITOGROWER®*

CONCLUSIONES

The product stored at 45ºC undergoes a slight degradation in the content of 6BAP, although the content remains within acceptable range. According to the guidelines of accelerated storage assay, it can be considered that if the product is stored following labelling directions (below 30ºC in a well ventilated area), the product will have a shelf life of at least two years.

Under the conditions of this study, the concentration of 6BAP in CITOGROWER® is reduced in 10,7% (absolute value) after two weeks of storage at 54ºC. When compared with the concentration after storage at room temperature and at 45ºC, it seems that degradation of cytokinins contained in CITOGROWER® is strictly a temperature mediated process.

REFERENCES

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