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Erratum to "Quantification of allantoin in various *Zea mays* L. hybrids by RP-HPLC with UV detection" [Pharmazie, 59(2004)524–527]

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Received December 12, 2007, accepted December 19, 2007

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Pharmazie 63: 550–551 (2008)
doi: 10.1691/ph.2008.7394

In 2004 a detection method for allantoin in Zea mays L. was proposed which contains a significant error regarding the identification of the analyte which is corrected here.

The code for our paper on Quantification of allantoin in various *Zea mays* L. hybrids by RP-HPLC with UV detection, published in volume 59 of this journal contains an error in the identification of analyte. We purposed to find a HPLC method for the determination of allantoin in corn silk. A literature study revealed the analysis of allantoin in this herb by RP-HPLC (Maksimovic et al. 2004). We tried to use this method and observed that the peak of acetone solvent has been mis-identified as allantoin in both the extract and standard solutions. That error is reflected below.

The chromatograms of silk extract and standard solution of allantoin (5 μg/ml) obtained following the above method are identical to the presented chromatograms by Maksimovic et al (Figs. 1 and 2). The UV spectrum of the peak at 4.7 min in the chromatograms of Figs. 1 and 2 was obtained by detector K-2600. The wavelength of absorption maximum was 266 nm. This method suggests the water-acetone (3 : 7 v/v) mixture for dissolving of the allantoin and extracting as solvent. For acquiring of HPLC chromatogram and UV spectrum of the solvent used in the sample preparation and standard solution, water of

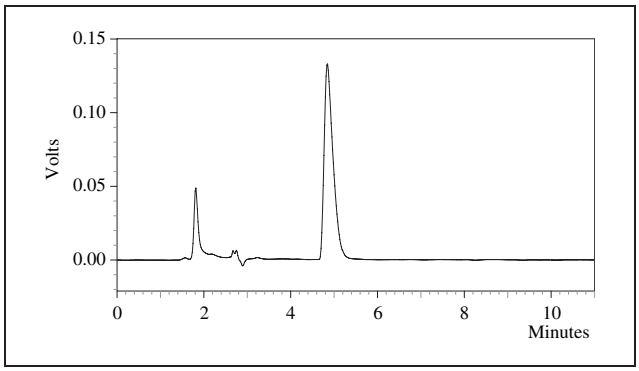

Fig. 1: Chromatogram of silk extract analyzed following the Maksimovic et al. method on an Econosil column at 235 nm

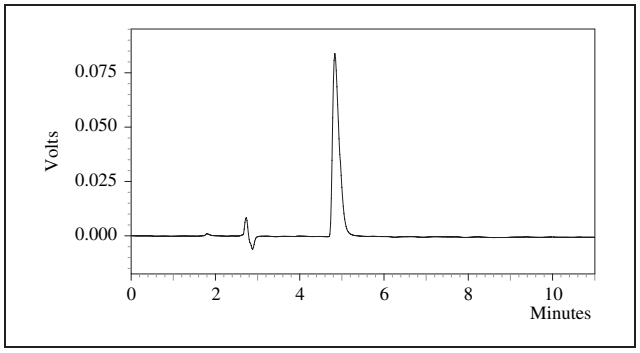

Fig. 2: Chromatogram of allantoin standard (5 μg/ml) with sample preparation solvent (aqueous solution of acetone 3.5% v/v) analyzed following the Maksimovic et al. method on an Econosil column at 235 nm

HPLC grade was mixed with acetone (3 : 7 v/v) then an aliquot of 1 ml of this solution diluted with water to 20 ml (acetone 3.5% v/v). A 20 μl volume of this solvent was injected into HPLC system. The acetone peak was observed at 4.7 min. Solution of aqueous acetone has absorption maximum close to 270 nm (Talrose et al. 2005) and it is an unfavorable solvent for UV chromatography due to strong absorbance at 235 nm in this method. It has been reported that, retention time of the allantoin peak is 4.68 min. We prepared the allantoin 500 μg/ml (100 times more concentrative) with solvent used in the sample preparation (aqueous acetone solution 3.5% v/v) and surveyed its HPLC chromatogram. The chromatogram showed two peaks at 2.7 and 4.7 min. UV spectrum of the peak at 2.7 min was obtained by spectrophotometer K-2600. Peak of allantoin standard solution (5 μg/ml) was not detected at 2.7 min due to low concentration and its poor absorbance at wavelength 235 nm. For proving of the peak at 2.7 min as allantoin, aqueous solution of allantoin (500 μg/ml) was injected into HPLC system and UV spectrum of allantoin recorded on detector K-2600. Allantoin peak was observed at 2.7 min but no peak detected at 4.7 min and the other times. The water, methanol, and water-methanol mixture were separately used for extraction of the sample as solvent instead of water-acetone mixture (3 : 7) and analyzed according to the mentioned method. No peak was observed during run time. Therefore, the peak at 4.7 min was known as acetone with respect to chromatograms of Figs. 1 and 2. Maksimovic et al. have mistakenly identified the peak of acetone as allantoin peak in chromatograms of Figs. 1 and 2. The ratio of plant sample-to-solvent (1 : 500) was negligible and separation and identification of the allantoin peak was impossible (Fig. 1). Allantoin was added to final extract and injected into HPLC system. The analyte peak was detected at 2.7 min. We used more concentrated extracts of silk for the analysis of analyte and observed no repeatability due to interference of peaks. This method used the triethylamine, sodium laurylsulfate and phosphoric acid for improvement of the peak tailing of allantoin. The symmetry of the allantoin peak was checked with below columns and methanol-water (20 : 80) as mobile phase without triethylamine, sodium laurylsulfate and phosphoric acid at ambient temperature. The peak shape of allantoin obtained with three kinds of columns was symmetrical, sharp and its retention time was pH independent but servers peak tailing and variation of retention time was observed for acetone. Acetone gives a strong hydrogen bond to the mobile phase. The high temperature of column (40 °C), weakens hydrogen bond and viscosity, improves the peak broadening and decreases its retention time relative to room temperature. Therefore, mobile phase, detection wavelength, extraction solvent and sample-to-solvent ratio used in this method were unfavorable and separation and identification of the analyte peak from the other peaks was found to be impossible in silk crude extract.

Experimental

1. Solvent and chemicals

Triethylamine, sodium laurylsulfate, orthophosphoric acid, acetone, methanol and water for chromatography (all from Merck) and allantoin (Sigma, A7878) with purity ≥98% were used.

2. Instrument and column

For acquiring of HPLC chromatogram and UV spectra of the analyte and acetone, separation was performed on a Knauer instrument (WellChrom, pump K-1001, fast scanning UV detector K-2600, analytical degasser K-5004, Injector 2301 with 20 μl loop). The analytical columns tested were Nucleosil 100 C_{18} (25 × 4.6 mm I.D., 5 μm particle size), Eurospher 100 C_{18} (25 × 4.6 mm I.D., 5 μm particle size) and Grace Econosil C_{18} (previous Alltech, USA; 25 × 4.6 mm I.D., 5 μm) without precolumn.

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