

Determination of Nitrite and Nitrate in Fruit Juices by UV Detection

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Introduction:

A method for the determination of nitrite and nitrate in parallel by UV detection is described. Simultaneous determination of nitrite and nitrate is proposed for monitoring threshold levels of these additives in foods. The acute toxicity of nitrate is in this instance of only peripheral importance. The primary health hazard lies rather in the rapid reduction of nitrate to nitrite which leads to the production of carcinogenic nitrosamine when nitrite reacts with secondary amines in the stomach. In addition, nitrite is also known to cause methemoglobinemia (oxygen deficiency) to occur in infants. In order to provide a way to monitor the legal recommended limits of these additives in foods, a rapid and cost-effective HPLC method is presented here.

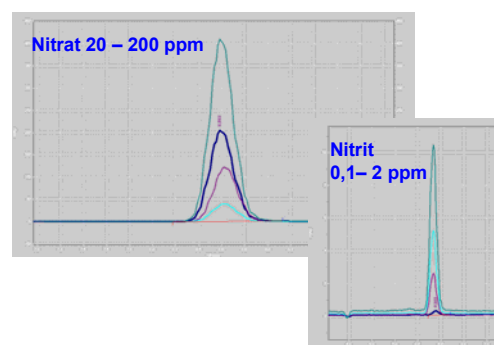
With an isocratic Smartline HPLC system from Knauer, one can carry out an uncomplicated and fast analysis. The analysis of nitrite and nitrate in fruit juices can be carried by ion pair chromatography on Knauer RP C18 phases using various counter ions (n-octylammonium or tetrabutylammonium). The detection takes place by UV at 210 nm. The Smartline system used consists of the Pump 1000, the UV Detector 2500, the Autosampler 3800, the Manager 5000 with degasser unit and the Column Oven 4000.

HPLC method parameters:

Column: Eurosil Bioselect 300-5 C18, 120 x 4 mm (I7Y413) [or Eurospher 100-5 C18, 250 x 4.6 mm (I115Y535)]
 Eluent: 0.01 M n-octylamine, set to pH 4 / 5 mM [or tetrabutylammonium hydrogen sulfate set to pH 6.5]
 Flow: 1 ml/min
 Temperature: 30 °C [40 °C]
 Injektion volume: 5 µl
 Detection: UV (Smartline 2500) at 210 nm

Sample preparation:

Carrez solution I: Solution of potassium hexacyanoferrate (II) in water, p.A. c = 150 g/L
 Carrez solution II: Solution of zinc sulfate in water, p.A., c = 300 g/L
 SPE method: condition SAX cartridges with 2 ml MeOH and 4 ml water, load 1 ml sample, wash with 3 x 1 ml water, elute with 2 ml 0.5 M NaCl solution



Calibration solutions were prepared from nitrite and nitrate standards in the range of 0.1 ppm to 25 ppm. For nitrate, the concentration range was extended to 200 ppm. Commercially available fruit and vegetable juices were investigated after treatment with the Carrez I and II solutions in a ratio of 50:1. Once precipitated, the sample was centrifuged and the supernatant was removed. In order to remove potentially interfering substances, the sample can be further cleaned on an SPE anion exchange cartridge. The filtrate can then be used directly for HPLC analysis.

Results:

Since both ions are typically present in markedly different concentrations, their simultaneous determination using a conductivity detector under a set sensitivity range is not always possible. Herein lies the strength of this method with ion pair chromatography and UV detection. With this technique, both ions can be detected down to a concentration of 0.1 ppm. This value represents the limit of quantitation for both ions. The use of two different ion pair compounds as eluents allows for adjustment of coeluting peaks and ensures reliable results. Linear calibration curves for the standards run with both eluents showed regression coefficients (r^2) of 0.9998 or better. The recovery rate of both ions is not influenced by the Carrez precipitation procedure: 95 – 98 %, without the SPE step, without the SPE step only reduces the recovery rate slightly, however the sensitivity is reduced by a factor of 2 through dilution in the elution step (1 ml loaded sample / 2 ml extraction volume). As had been expected, nitrite ion concentrations below the 0.1 ppm limit of quantitation were found in the fruit and vegetable juices examined. Manufacturers of such juices must not allow nitrite concentrations to exceed 0.1 ppm in their products. Nitrate values varied widely among the vegetable juices tested. While the nitrate value for one brand of carrot juice was 140 ppm, another brand of red beet juice gave a nitrate value of approximately 850 ppm, well outside of the calibration range.

