

## **Abstract**

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Bc. Ivana Stachová

Supervisor: Doc. RNDr. Dalibor Šatínský, Ph. D.

Title of diploma Thesis: HPLC method development for artificial colorants determination in green beer samples

HPLC method was used and validated for the simultaneous determination of synthetic water-soluble dyes: E 102 - Tartrazine, E 104 - Quinoline Yellow, E 110 - Sunset Yellow, E 131 - Patent blue, E 132 - Indigo carmine, E 142 - Green S, E 133 - Brilliant Blue FCF and E 143 - Fast Green FCF. The method was applied for direct determination of these dyes in samples of green beers Jarní pivo 11° (Primátor, Náchod), Krasličák 14° (Ježek, Jihlava), Zelený král Vratislav 12° (Konrád, Vratislavice), Velikonoční speciál 14° (Starobrnno, Brno), Velikonoční ležák 12° (Radniční pivovar, Jihlava) Rohozec 11° (Rohozec, Malý Rohozec), Zelená nefiltrovaná 12° (Žlebské Chvalovice), Velikonoční zelený ležák 12° (Purkmistr, Plzeň), Velikonoční kopřivová 12° (Modrá Hvězda, Dobřany), Valášek 12° (Valášek, Vsetín), Podkovář 12° (Podkovář, Dolní Cetno).

Analytical Chromolith Performance CN 100 x 4.6 mm column and guard column Chromolith CN 5 x 4.6 mm Merck were used and mobile phase contained 40% (v/v) methanol / 2% (v/v) acetic acid buffer with addition of ammonia for pH adjustment to value 7.0. Successful separation was obtained for all the compounds using an optimized gradient elution within 12 minutes. Analysis was carried out at temperature of 30°C and the flow rate 2 ml/min, the injection volume was set at 10 µl.

The DAD detector was used for monitoring the dyes at the 3 wavelengths 420 nm ((Tartrazine a Quinoline yellow), 482 nm (Sunset yellow) a 625 nm (Indigo carmine, Green S, Brilliant blue FCF, Patent blue a Fast green FCF).

Almost all of the analyzed samples contained Brilliant blue, except Zelený král Vratislav 12° (Konrád, Vratislavice), Velikonoční kopřivová 12° (Modrá Hvězda, Dobřany), Podkováň 12° (Podkováň, Dolní Cetno). There was found no artificial colorant. Moreover, in samples Velikonoční ležák 12° (Radniční pivovar, Jihlava), Zelená nefiltrovaná 12° (Žlebské Chvalovice), Velikonoční zelený ležák 12° (Purkmistr, Plzeň), Valášek 12° (Valášek, Vsetín) was found Tartrazine.

Keywords: HPLC, E 102 - Tartrazine, E 104 - Quinoline yellow, E 110 - Sunset yellow, E 131 - Patent blue E 132 - Indigo carmine, E 142 - Green S, E 133 - Brilliant blue FCF and E 143 - Fast green FCF, synthetic dyes, beer