P-0405-M OPTIMIZATION AND VALIDATION OF AN ANALYTICAL METHOD FOR DETERMINATION OF GLYPHOSATE AND AMPA IN APPLES USING SPE-HPLC WITH POST-COLUMN DERIVATIZATION. Juliano de A. Andrade[1]; Sonia Claudia do N.de Queiroz[2]; Marley M.Tavares[2]; Isabel Cristina S. F.Jardim[1]; [1]Instituto de Química, UNICAMP, Campinas, Brazil; [2]EMBRAPA Meio Ambiente, Jaguariuna, Brazil

Glyphosate (N-(phosphonomethyl)glycine) is a non-selective, broad spectrum, post-emergent herbicide with systemic activity in plants. The EPA has set a maximum residue limit (MRL) for glyphosate in fruit in the range of 0.2-5 mg/kg. The FAO has set a MRL for glyphosate in the range of 0.1-5 mg/kg for fruits. In Brazil, the Agência Nacional de Vigilância Sanitária (ANVISA) established the MRL values for glyphosate in fruits at 0.2 mg/kg. Recent papers reveal that glyphosate has been found in various foods, especially in fruits such as apples at high mg/kg levels. Glyphosate is a very polar compound that must be assayed together with aminomethylphosphonic acid (AMPA), the main metabolite of glyphosate. Because of their high polarity, low volatility and low mass adequate rapid methodologies to determine these compounds at the sub-µg/L level in aqueous and food samples have not been reported. Most methods for determination glyphosate and AMPA are very laborious, involving clean-up step before chromatographic analysis. In this paper, a method to determine glyphosate and AMPA using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with fluorescent detection was developed and validated. Glyphosate was oxidized with sodium hypochlorite (bleach) at 38 oC. The product from the oxidation reaction (glycine) and AMPA were then separated using an Aminex glyphosate analytical column (300 x 4.6 mm) and guard column (100 x 4.6 mm) with a mobile phase containing 5 mmol/L of potassium dihydrogenphosphate in methanol at pH 2.1, adjusted with H3PO4. After separation, post-column derivatization with o-phthalaldehyde and 2-mercaptoethanol gave highly fluorescent derivatives, which were detected using excitation at 339 nm and emission at 445 nm. Standard stock solutions (1000 µg/mL) of glyphosate and AMPA were prepared with HPLC-grade water. Mixed standards (0.005 – 1 µg/mL) were prepared in mobile phase for construction of the analytical curves that were linear between 0.01 µg/mL and 1 µg/mL for glyphosate and 0.02 µg/mL and 1 µg/mL for AMPA, with excellent correlation coefficients (> 0.9999, n = 12) without visible bias. The limits of detection (LOD) for glyphosate and AMPA were 0.005 µg/mL and 0.006 µg/mL, respectively, well within the established limits. The method developed is rapid, simple, accurate, sensitive and selective for determination of these polar pesticides and was applied to determine the presence of these compounds in apples.