

Development and validation of a novel HPLC-PDA method for the detection of preservatives in milk

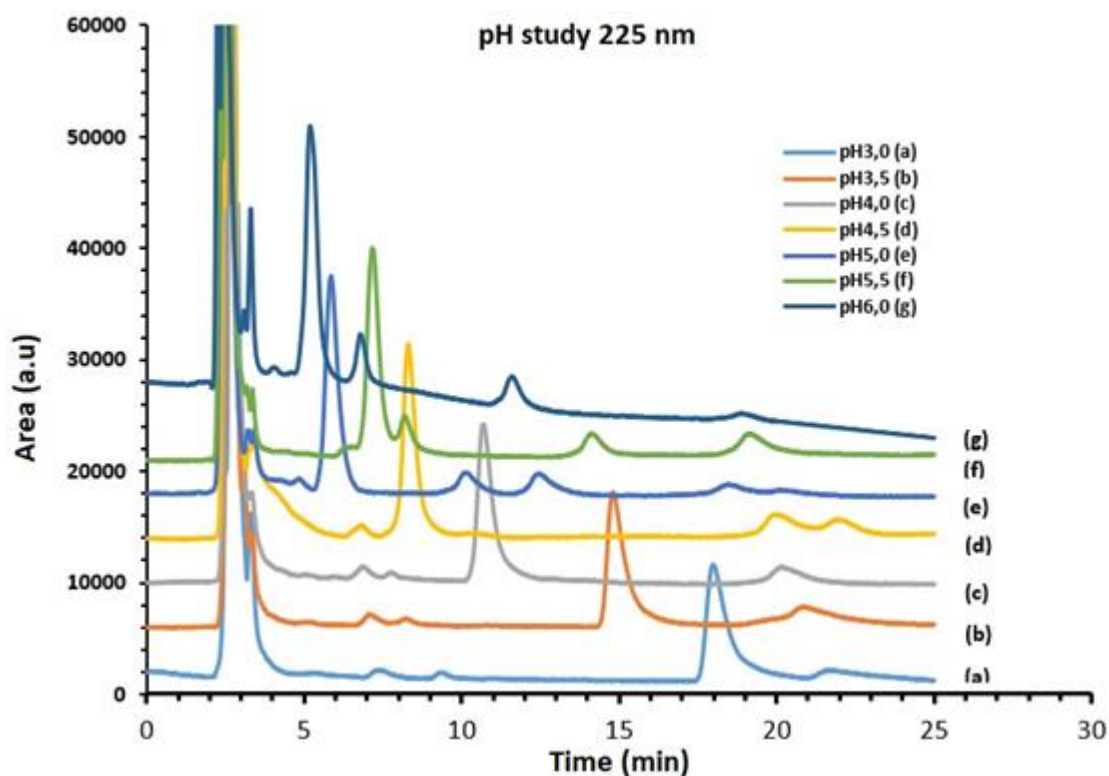
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SUPPLEMENTARY FILE

The primary objective of this study was to determine the optimal pH value for the proposed method, with a focus on achieving the highest resolution between the peaks of the preservatives sodium benzoate and potassium sorbate at a concentration of 5.00 mg L⁻¹. Different pH values were studied ranging from 3.0 to 6.0 with a 0.5 interval, as shown in Figure S1.

Supplementary Figure S1

Chromatographic separation according to the pH of the mobile phase for preservatives sodium benzoate and potassium sorbate, both at a concentration of 5.00 mg L⁻¹.



Supplementary Figure S2

Chromatograms of the robustness evaluation of the chromatographic method in relation to the column temperature variation for a milk sample with sodium benzoate and potassium sorbate standards, both at concentration of 10.00 mg L^{-1} at 225 nm and 255 nm.

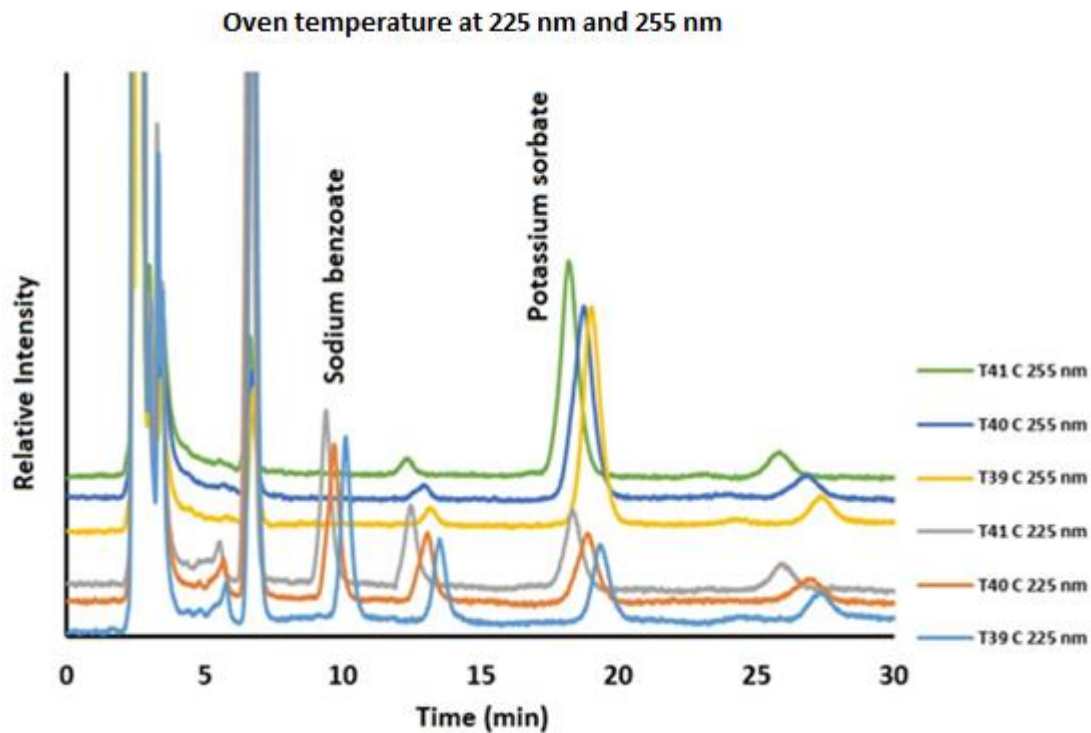


Chart S1:

Comparison between the novel and the official methods for the quantitation of preservatives (sodium benzoate and potassium sorbate) through HPLC, regarding reagents, equipment, processes, and duration time.

	Validated novel method	Official method (ISO 9231, 2008)
Sample volume	1.0 mL	100 to 3000 mg
Extraction process	1.0 mL of the sample and 1.0 mL of Acetonitrile (ACN) Homogenize in vortex (1 min) Microtube centrifuge (10 min – 10,000 rpm) Filtration in a 0.22 µm PTFE membrane	Sodium hydroxide 25 mL Sulfuric acid Potassium Hexacyanoferrate 2 mL Zinc acetate 2 mL Methanol 40 mL Ultrasonic Bath or 70°C Water Bath (15 min) pH meter Hydrophilic filter 0.45 µm
Extraction time (min)	12	60
Mobile Phase composition (MP)	Acetonitrile (12% vol.) Phosphate buffer (88% vol.) pH meter	Methanol (10% vol.) Phosphate buffer (90% vol.) pH meter Hydrophilic filter 0.45 µm
MP flow rate (mL min⁻¹)	1.0	1.2
HPLC runtime (min)	25	10
Injection volume (µL)	10	5 to 20
Sodium benzoate (λ_{max} - nm)	225	227
Potassium sorbate (λ_{max} - nm)	255	250
Benzoate QL	>0.618 mg L ⁻¹	>5 mg kg ⁻¹
Sorbate QL	>0.328 mg L ⁻¹	>5 mg kg ⁻¹

λ_{max} : maximum wavelength used for detection; QL: quantitation limit.