

## VAC\_17 - Development of an analytical method using HPAE-PAD for lactose determination in a stabilizer solution used in the production of vaccines

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**Introduction:** Stabilizing adjuvants plays an important role in vaccine formulation. They make it possible for some preparations to resist longer to higher temperatures without losing efficacy. Lactose is a disaccharide composed of galactose and glucose condensed by  $\beta$ -1 $\rightarrow$ 4 glycosidic linkage. It works as a part of formulation as a stabilizing agent in a wide scope of vaccines produced by Bio-Manguinhos/FIOCRUZ such as HiB (*Haemophilus influenza* B), Meningococcal AC and MMR (measles, mumps, rubella). It is important to control lactose concentration in formulations for quality control purposes. In this context, High-Performance Anion- Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) is a powerful technique to determine carbohydrates in pharmaceutical products, offering advantages such as accuracy and selectivity with minimal sample preparation, cost-effectiveness, and rapid analysis compared to other analytical techniques.

**Objectives:** Develop an analytical method using HPAE-PAD for determination of lactose in 15,75% (w/v) stabilizer solution for vaccine production.

**Methodology:** This study used an Ion Chromatograph Professional IC Vario 940 Methrom, with a Pulse Amperometric Detector, a CarboPac<sup>TM</sup> PA10 column, and a BorateTrap<sup>TM</sup> guard column. The mobile phase was sodium hydroxide 100 mM. A five-point standard curve was prepared, covering a concentration range from 0.5 to 10.0 ppm. Subsequently, the method was tested for its linearity, precision, and accuracy, by RDC166/17. Lactose USP standard and six independently prepared solutions containing 15.75% (w/v) lactose as a stabilizer were employed to check method performance. The following acceptance criteria were applied: R<sup>2</sup> value greater than or equal to 0.99, recovery rates within 80% and 120%, and a Relative Standard Deviation (RSD%) below 10%.

**Results:** The method demonstrated linearity with a correlation coefficient value of 0.9998. The triplicate preparations of the calibration curve exhibited RSD% values ranging from 3.3 to 8.79%. Analyst 1 and 2 provided RSD% values, in precision study, of 7.2 and 3.0, respectively, while the intermediate precision showed a 6.5% of RSD%. The recovery values for the six individual determinations ranged from 98 to 116%.

**Conclusion:** The proposed method for quantitation of lactose in 15,75% (w/v) stabilizer solution by HPAE-PAD shows good precision, linearity, and accuracy and it is suitable for analytical validation. Due to its applicability to Lactose, the HPAE-PAD technique could be used for the determination of other saccharides with importance for vaccine development and production.

**Keywords:** Lactose; HPAE-PAD; Stabilizers