HPLC Analysis of Penicillins in Veterinary Drugs

Samanidou V* and Evaggelopoulou EN

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Penicillins are beta (β)-lactam antimicrobial which are used against various organisms by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. Broad-spectrum penicillins include ampicillin (AMP), penicillin G (PG), penicillin V (PV), oxacillin (OXA), cloxacinil (CLO) and dicloxicillin (DICLO). They act as bactericides being effective against Gram-positive and Gram-negative bacteria, but not very effective against Pseudomonas. Their chemical structures are shown in Figure 1.

An HPLC method was determined and validated for the simultaneous determination of penicillins in veterinary drugs, using an LC-10AD VP HPLC pump with Photodiode Array Detector, in compliance with data acquisition software LabSolutions-LC solutions by Shimadzu. Separation was performed on an Inertsil, C18, 250 x 4 mm, 5 μm analytical column purchased from MZ-Analysentechnik (Mainz, Germany). Mobile phase consisted of CH3COONH4 0.05M and acetonitrile at a volume ratio 85:15 remaining isocratic for 5 min and changing to 40:60 in the next 20 min, was delivered at a flow rate of 1 mL/min. The method was applied to the analysis of commercially available pharmaceuticals.

Two veterinary drugs were analysed: ORBENIN L.A. by Pfizer Italia Srl (Latina, Italy), which contains 200 mg cloxacinil per 3 g of product and CLOXALENE PLUS by FatroS.p.A. (Bologna, Italy) containing 11 g ampicillin and 5 g dicloxicillin per 1 mL of product [1-4].

Accuracy of the method for analysed veterinary drugs ranged from -4.9.3% expressed as relative error. A representative chromatogram is shown in Figure 2.

**Figure 1:** Chemical structures of penicillins

**Figure 2:** Chromatogram of Cloxalene Plus injection (4.4 ng/μL Lampicillin (5.882 min) and 2 ng/μL dicloxicillin (21.603 min) in presence of lamotrigine as internal standard (14.133 min)

References


*Corresponding author: Samanidou V, Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, Tel: +302310976098; Fax: +302310997719; E-mail: samanidou@chem.auth.gr

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