

ABSTRACT

The current study was carried out firstly to develop an analytical method for the detection of most commonly used group of antibiotics i.e., Beta Lactam by using reversed-phase HPLC equipped with PDA detector in buffalo raw milk available at district Lahore. The second main purpose was to develop a protocol that is sensitive enough, not only to detect but also to quantify the accurate amount of a particular penicillin & cephalosporin in raw milk samples. One hundred and fifty samples of raw buffalo milk were collected from District Lahore, to detect and quantify the accurate amount of residue of Beta-Lactam antibiotics found in them. Initial screening of samples was done by using Microbial Inhibition Assay i.e. (*Bacillus subtilis* Qualitative field Disc Assay). Majority (99) of samples (66%) showed a negative result for the presence of Beta Lactam antibiotic residues in them i.e. no visible transparent zone was formed around disc. While 54 samples (34%) were found positive with zone size of 1-11mm.

54 Milk samples detected positive in screened test were confirmed for β - lactam antibiotic residues through High Performance Liquid Chromatography (HPLC method). The quantification of analytes was limited to Penicillin G, Amoxicillin and Ampicillin. Due to unavailability of other beta lactams standards at the time of study only three were measured and quantified in the present study. The result shows that over the 54 samples analyzed, 14 (27.45%) were contaminated with Penicillin-G residues, 20 (39.21%) with Amoxicillin, and 20 (39.21%) with Ampicillin. Penicillin G residues in raw milk ranged between 6.83 to 491.2 $\mu\text{g/L}$ and averaged $130.07 \mu\text{g/L} \pm 157.3 \mu\text{g/L}$. Amoxicillin residues in raw milk varied between 36.6 and 1592.3 $\mu\text{g/L}$ and averaged $437.71 \mu\text{g/L} \pm 476.5 \mu\text{g/L}$. Ampicillin residues level in the raw milk samples ranged between 26.1 to 352.83 $\mu\text{g/L}$ and averaged $133.43 \mu\text{g/L} \pm 82.4 \mu\text{g/L}$.

In positive raw milk samples, Amoxicillin's residue has been detected in highest concentration (mean= $437.7 \mu\text{g/L}$) followed by Ampicillin's (mean= $133.43 \mu\text{g/L}$) and Penicillin-G (mean= $130.07 \mu\text{g/L}$). In positive samples Amoxicillin's concentration (mean= $437.7 \mu\text{g/L}$) has been calculated as 109.42 and 43.77 times, Ampicillin's residue (mean= $133.43 \mu\text{g/L}$) is 33.35 and 13.34 times, while Penicillin-G's concentration

(mean=130.07 μ g/L) has been detected as 32.51 and 26.01 times higher than EU (4 μ g/L) and FDA standard MRL values (10 μ g/L and 5 μ g/L) respectively.

At the end, a profound and precisely modified HPLC- PDA technique was devised for quantitative detection of three Beta Lactams (penicillin) from raw buffalo milk at District Lahore. This method was developed first time in Pakistan. Results of present study reveals that residual concentrations of Amoxicillin and Ampicillin in raw milk was dominant as compared to Penicillin-G. Remarkably higher level of Amoxicillin, Ampicillin and Penicillin-G residues in raw milk indicates a major contrast to Maximum Residual Limits (EU and FDA standard) revealing that improper use of these antibiotics could be incurred in the vicinity of District Lahore.