Hydrophilic-Interaction Planar Chromatography in Ultra Sensitive Determination of \( \alpha \)-Aminocephalosporin Antibiotics. Application to Analysis of Cefalexin in Goat Milk Samples Using Modified QuEChERS Extraction Technique

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Abstract:

A highly sensitive, selective and precise HPTLC method coupled with fluorescence detection was developed and validated for the determination of \( \alpha \)-aminocephalosporin antibiotics; namely cefalexin, cefadroxil and cefradine in their standard solutions. The applicability of the developed methodology was demonstrated via analysis of cefalexin in goat milk samples. Full optimization of the fluorescence derivatization reaction was carried out with regard to the standard solutions of the studied compounds or after extraction of milk samples. The separation of the studied compounds was performed on HPTLC precoated silica gel plates 60 F254 using acetonitrile: water in a ratio 85:15 (v/v) as a mobile phase. The retention behavior of the formed derivatives was discussed in detail. It was found that hydrophilic interaction mode is the main interaction mechanism governing the retention of the formed derivatives. In addition, an experimental design approach was conducted for optimization of the chromatographic conditions. Modified QuEChERS was applied as an efficient extraction technique of cefalexin from both spiked and real goat milk samples. Optimization of QuEChERS extraction technique to achieve the highest extraction recovery was performed and the results indicate that this method provides a good extraction recovery (83\%\%–116\%) for cefalexin from goat milk samples. Limit of detection (LOD) of the developed method was found to be 0.023, 0.005, and 0.023 ng band\(^{-1}\) for cefalexin, cefadroxil and cefradine, respectively in their standard solutions and 0.165 ng band\(^{-1}\) for cefalexin in goat milk samples. According to the achieved LOD values, the method sensitivity was quasi-equivalent to other methods based on expensive techniques such as HPLC-UV and HPLC-MS and it is sufficient to determine cefalexin below its MRL in milk samples. Moreover, the method was successfully applied to a pharmacokinetic study of cefalexin in goat milk after single intramuscular injection of 10 mg of cefalexin kg\(^{-1}\) per body weight.

Keywords:

Hydrophilic-interaction planar chromatography, Fluorescamine, Pre-chromatographic derivatization, Modified QuEChERS, \( \alpha \)-Aminocephalosporin antibiotics, Pharmacokinetic study

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