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HPLC determination of theophylline and paracetamol in fresh and powdered milk

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Introduction

Theophylline and paracetamol are drugs worldwide used both in human and veterinary medicine. Paracetamol (or acetaminophen) is employed as analgesic and antipyretic, and theophylline is a bronchodilator drug used in the treatment of asthma and chronic obstructive pulmonary disease. Both drugs are extensively used in pediatrics and their dosage is based on the child's body weight. Due to possible liver toxicity (paracetamol) and narrow therapeutic index (theophylline) it is imperative to carefully select and monitor the dose administered to children. Additionally, the recent proposal of milk as a platform to deliver drugs in pediatrics [1] has prompted the development of a simple and sensitive high-performance liquid chromatography (HPLC) quantification method, of both theophylline and paracetamol, in the complex matrix that milk represents.

Methods

A Merk® liquid chromatography system, model Hitachi Lachrome, equipped with a L-7400 UV-vis detector, a quaternary pump, a manual injector Rheodyne 7750i, and a data system (D-7000 HSM software) were used. A Merk® analytical Purospher C18 5 µm guard column (4 x 4 mm internal diameter) and a Purospher C18 5 µm column (250 x 4 mm internal diameter) were used for the chromatographic separation of paracetamol and theophylline, at room temperature. *p*-Aminobenzoic acid (PABA) was used as an internal standard (IS). Chromatography was carried out under isocratic conditions with a 10 mM ammonium acetate buffer: acetonitrile: methanol (90:5:5 v/v/v) pumped at a 1 ml/min flow rate. The detector wavelength was set at 272 nm and the run time was 14 min. The HPLC method was validated with respect to specificity, linearity, accuracy, precision and recovery, according to ICH guidelines [2]. Fresh and powdered commercial milks were used to produce solid (minitablets and spray-dried powders) and liquid pharmaceutical formulations containing theophylline or paracetamol. Fresh milk (used as a control) and drug-milk solutions (0.1 ml) were spiked with 0.1 ml of IS (100 µg/ml solution) and diluted with ultrapure water to a final concentration within the linear range of the method. If the starting material was in the solid state the sample was suspended/dissolved by vortexing, in ultrapure water and treated as described before. The mixtures were then centrifuged (2000 rpm/10 min) and 20 µl of the supernatant was injected into the HPLC system and analyzed in triplicate.

Results and Discussion

The concentration of standard drug solutions ranged from 0.2 to 50 μg/ml, established later on as the linear range of the method. HPLC retention times for paracetamol and theophylline were respectively 10.6 and 11.8 min. Calibration curves were constructed using the peak area ratios of drug + IS *versus* the corresponding drug concentration by least-squares linear regression. Sample concentration was calculated from the resulting regression equations. Calibration curves typically showed squared correlation coefficients (r^2) of 0.999. The specificity of the method was confirmed since it was capable of accurately measure the drugs without interference from milk components. Accuracy and precision were assessed by repeated analysis (n=6) of the drugs + IS control samples at three drug concentrations (0.5, 5 and 25 μg/ml). The average percentage recovery (99.2±7.5%) was calculated by comparing the mean peak area of these three control milk solution samples, with those obtained in water. The limit of quantification (LOQ) was approximately 0.1 μg/ml and the limit of detection (LOD) was 0.05 μg/ml. Repeatability and intermediate precision were assessed by six injections at each of three theophylline concentrations (0.5, 5 and 25 μg/ml) respectively on the same day (intraday) and in 5 different days (interday).

Conclusion

In this work, a sensitive, selective, accurate and inexpensive HPLC method for determination of paracetamol and theophylline in fresh and powdered milk was developed and validated. The method can be applied for the pharmaceutical development of milk-containing dosage forms for pediatric use, as well as in the quantification of residues of these drugs in the milk of breast-feeding mothers and livestock, with potential application both in the pharmaceutical and food industries.

References:

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