

## INTRODUCTION

Theophylline and paracetamol are drugs used worldwide 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

### Chromatography

A Merck® 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 in a Merck®-Hitachi Lachrome liquid chromatography system, equipped with a L-7400 UV-vis detector, a quaternary pump, a column oven, a manual injector Rheodyne 7750i and a data system (D-7000 HSM software). PABA (*p*-Aminobenzoic acid) 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 and samples quantified at 272 nm. The concentration of standard drug solutions ranged from 0.2 to 50 µg/ml, established later on as the linear range of the method. The HPLC method was validated with respect to specificity, linearity, accuracy, precision and recovery, according to ICH guidelines [2].

### Sample preparation

Fresh and powdered commercial milks were used to produce solid (minitables and spray-dried powders) and liquid (solutions, emulsions) 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 (100µg/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. 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

HPLC **retention times** for paracetamol and theophylline were respectively 10.6 and 11.8 min (Fig.1). **Calibration curves** were constructed using the peak area ratios of drug + IS *versus* the corresponding drug concentration by least-squares linear regression, with typical correlation coefficients ( $r^2$ ) of 0.999 (Fig.2). Sample concentration was calculated from the resulting regression equations. The **specificity** was confirmed by the chromatograms (Fig.1), since the method 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 ( $99.2\pm7.5\%$ ) 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 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).

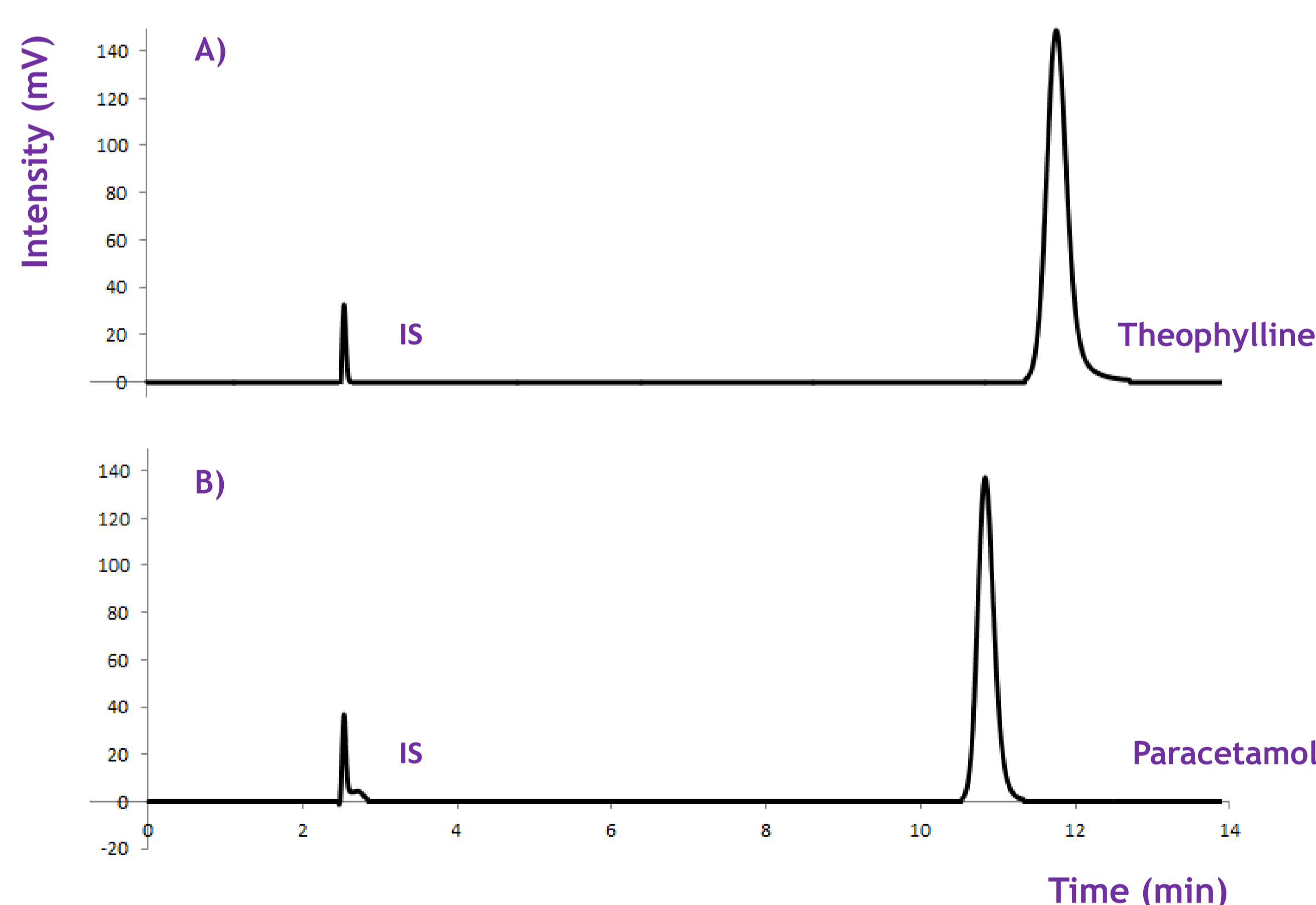


Fig. 1. Chromatograms of milk samples spiked with 100µg/ml of theophylline and IS (A) and 100µg/ml of paracetamol and IS (B).

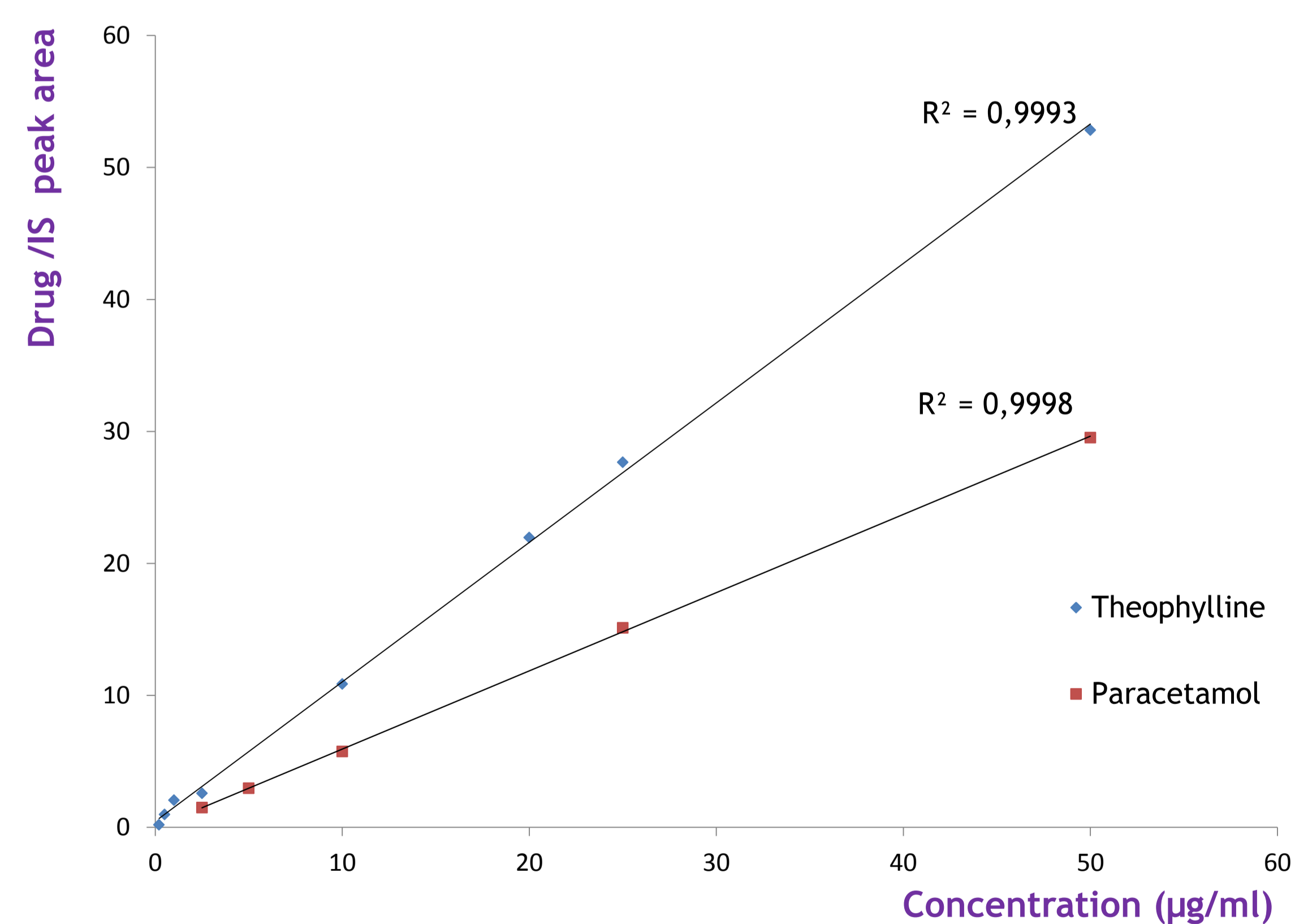


Fig. 2. Typical calibration curves of theophylline and paracetamol. Each curve is based on eight calibration standards with triplicate injections.

## 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

- [1] Pinto J. T. *et al.* (2015) Evaluation of the ability of powdered milk to produce mini-tablets containing paracetamol, *1st European Conference on Pharmaceutics*, 33.
- [2] International Conference on Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use (2005) *Validation of analytical procedures: text and methodology* Q2(R1), 1.

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