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Short communication

Determination of cisapride and norcisapride in human plasma using high-performance liquid chromatography with ultraviolet detection

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Abstract

A simple, rapid and reproducible high-performance liquid chromatographic assay for cisapride and norcisapride in human plasma is described. Samples of plasma (150 μl) were extracted using a C_{18} solid-phase cartridge. Regenerated tubes were eluted with 1.0 ml of methanol, dried, redissolved in 150 μl of methanol and injected. Chromatography was performed at room temperature by pumping acetonitrile–methanol–0.015 *M* phosphate buffer pH 2.2–2.3 (680:194:126, v/v/v) at 0.8 ml/min through a C_{18} reversed-phase column. Cisapride, norcisapride and internal standard were detected by absorbance at 276 nm and were eluted at 4.3, 5.3 and 8.1 min, respectively. Calibration plots in plasma were linear ($r>0.998$) from 10 to 150 ng/ml. Intraday precisions for cisapride and norcisapride were 3.3% and 5.4%, respectively. Interday precisions for cisapride and norcisapride were 9.6% and 9.0%, respectively. Drugs used which might be coadministered were tested for interference.

Keywords: Cisapride; Norcisapride

1. Introduction

Cisapride is widely used for the treatment of some gastrooesophageal diseases caused by disordered motility [1,2]. Conditions that lead to increase in plasma levels of cisapride, such as drug overdose, liver disease or concomitant treatment with drugs that interfere with hepatic metabolism (e.g. macrolide antibiotics and ketoconazole) or limited cytochrome P-450 function reported in neonates, can caused Q–T prolongation [3,4]. In human plasma and urinary excretion, norcisapride is major metabolite governed by its formation from N-dealkylation of cisapride [5]. Norcisapride is one sixth as active as the parent drug [6]. However, the elimination of this metabolite is impaired with renal failure associated with an apparent halflife three to four times longer and with ratios of norcisapride to cisapride higher than those reported in healthy volunteers [6]. The accumulation of norcisapride and its potential to produce cardiac toxicity has not been explored and remains unknown although it is structurally related to procainamide. Our initial work involved the determination of cisapride and its metabolite, norcisapride, in human plasma using a reversed-phase HPLC and a liquid–solid extraction adapted for analysis of low plasma volumes. Published methods for the determination of plasma cisapride concentrations use fluorescence detection or too large plasma volume (2 ml) and did not quantify norcisapride [7,8]. We report a

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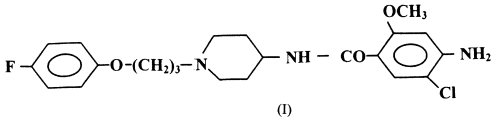
simple, rapid and reproducible method quantifying both cisapride and norcisapride by UV detection.

2. Experimental

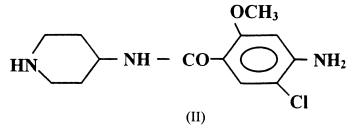
2.1. Materials and reagents

Cisapride, norcisapride and risperidone (internal standard) were generously given by Janssen–Cilag (Beerse, Belgium). Their chemical nomenclature and structure are shown in Fig. 1. Sodium hydrogen phosphate, disodium hydrogen phosphate and orthophosphoric acid 85% were supplied by Sigma (Isle d'Abeau Chesnes, France), Carlo Erba (Nanterre, France) and Prolabo (Fontenay-sous-Bois, France), respectively. All solvents and chemicals used were HPLC or analytical reagent grade and no further purification was carried out. The HPLC system (Waters, Millipore, Milford, MA, USA) consisted of a pump (Model 501) connected to a 100 μl Rheodyne valve (Model 7125, Touzart and Matignon, Vitry-sur-Seine, France), a spectrophotometric detector (Model LC) set at 276 nm and a integrator system (Model 545). The HPLC column (NovaPak, Waters) was a C_{18} reverse-phase, 4 μm particle size 3.9 mm×150 mm column run at ambient temperature. The mobile phase was acetonitrile–methanol–0.015 *M* phosphate buffer pH 2.2 (68:19.4:12.6; v/v/v) and was pumped at a flow-rate of 0.8 ml/min. The composition of buffer was 0.620 g of sodium hydrogen phosphate, 0.22 ml of orthophosphoric acid 85% and water for irrigation to 500 ml. The control plasma was obtained by our department of transfusion and was collected into a polyethylene bag without additives.



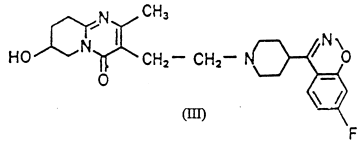
(I)

Cis-4-amino-5-chloro-N-(1-(3-(4-fluorophenoxy)-propyl)-3-methoxy-4-piperidinyl)-2 methoxybenzamide



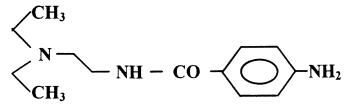
(II)

Cis-4-amino-5-chloro-N-(3-methoxy-4-piperidinyl)-2-methoxybenzamide



(III)

3-(2-(4-(6-fluoro-1,2-benzisoxazole-3-yl)-piperidino)ethyl)-6,7,8,9-tetra-hydro-2-methyl-4H-pyrido (1,2-a) pyrimidine-4-one



(IV)

4-amino-N-[2-(di-ethylamino)]ethylbenzamide

Fig. 1. Chemical nomenclature and structure of cisapride (I), norcisapride (II), risperidone (III), and procainamide (IV).

2.2. Standard and sample preparation

Standard solutions of cisapride and norcisapride were prepared in methanol at 100 mg/ml. A stock solution of the internal standard, risperidone, was prepared at 1 μg/ml in methanol. To obtain the calibration curves of cisapride and norcisapride, standard solutions were prepared in plasma to achieve 10, 25, 50, 100 and 150 ng/ml. For the assay of plasma and standards samples, 200 μl of plasma was mixed with 50 μl of methanol, 50 μl of internal standard and 700 μl of phosphate buffer (pH 7.9, 0.05 *M*) in a 10 ml glass tube. The tube was centrifuged at 1500 *g* for 10 min. The supernatant was transferred to a 1 ml LC-18 extraction tube (Supelclean, Supelco, Bellefonte, PA, USA) which had been preconditioned with 2 ml of methanol, 1 ml of water and 1 ml of phosphate buffer (pH 7.9, 0.05 *M*). The extraction tube was washed with 800 μl of phosphate buffer, and elution was effected with 1 ml of methanol. The methanol was evaporated to dryness under a stream of nitrogen in a metal heating-block at 40°C. The residue was dissolved with 150 μl of methanol and injected onto the column. The potential interference caused by common drugs was checked by analysis of drug supplemented plasma.

3. Results

Fig. 2 shows a chromatogram of a blank control plasma, control plasma spiked with cisapride and norcisapride at various concentrations and a child's plasma. The retention times of cisapride, norcisapride and internal standard were 4.3 (k=2.7), 5.3 (k=3.7) and 8.1 (k=6.5) min, respectively. The extraction efficiencies for cisapride at 50 and 100 ng/ml were 64.7±4.9% and 62.9±1.1%, respectively. The extraction efficiencies for norcisapride at 50 and 100 ng/ml were 48.4±2.2% and 46.5±3.8%, respectively. Increasing the extraction pH, the recovery of cisapride was higher reaching 85% but in contrast to a decrease in norcisapride recovery.

3.1. Calibration of cisapride after extraction

The standard curves were analysed by unweighted linear regression. The following values for slope, intercept and correlation coefficient, respectively, were calculated: 0.0176±0.07, 0.0757±0.0042, 0.998±0.0034 (mean±S.D.; n=5). The intercept was tested for difference from zero using a t-test at α=0.05 and was found not to be significantly different from zero.

3.2. Calibration of norcisapride after extraction

The standard curves were analysed by linear regression. The following values for slope, intercept and correlation coefficient, respectively were calculated: 0.0108±0.023, 0.0655±0.0033, 0.998±0.0041 (mean±S.D.; n=5). The intercept was tested for difference from zero using a t-test at α=0.05 and was found not to be significantly different from zero.

3.3. Limit of quantification

The lowest standard concentrations of cisapride and norcisapride were both 10 ng/ml.

3.4. Intraassay precision

Five replicates each of different concentrations of cisapride and norcisapride in plasma were analysed in a single run, independently of samples from calibration curves. The variability, expressed as relative standard deviation (R.S.D.), was 1.2, 3.2, 3.3, 2.2 and 1.6% for the 10, 25, 50, 100 and 150 ng/ml cisapride plasma samples, respectively. The precision as R.S.D. was 2.6, 5.4, 0.4, 4.3 and 0.95% for 10, 25, 50, 100 and 150 ng/ml norcisapride plasma samples, respectively.

3.5. Interassay precision and accuracy

Single plasma samples of cisapride and norcisapride (10 and 150 ng/ml) were analysed in five different analytical runs. Samples were prepared daily, independently of samples from calibration curves. Precision expressed as R.S.D. was 9.6 and 1.9% for 10 and 150 ng/ml cisapride, respectively. Precision as R.S.D. was 9.0 and 1.6% for 10 and 150 ng/ml norcisapride, respectively. The accuracy, for samples expressed as percent difference from nominal (%DFN), was calculated as %DFN=[(mean−nominal)/nominal]×100. For cisapride, the DFN was −17.7, 1.9 and −1.6% for 10, 50 and 150 ng/ml, respectively. For norcisapride, the DFN was 13.4, 0.4 and 1.4% for 10, 50 and 150 ng/ml, respectively.

3.6. Selectivity

Different drugs were injected onto the column after the specified extraction chromatographic and using the conditions as described. Domperidone, metopimazine and trimebutine interfered with the cisapride peak and ketotifen with the norcisapride peak. No tested drugs interfered with the internal standard.

4. Discussion

The HPLC determination of cisapride has been studied by several authors [3,8]. The previous HPLC methods did not determine the major cisapride metabolite, norcisapride. The Woestenborghs et al.

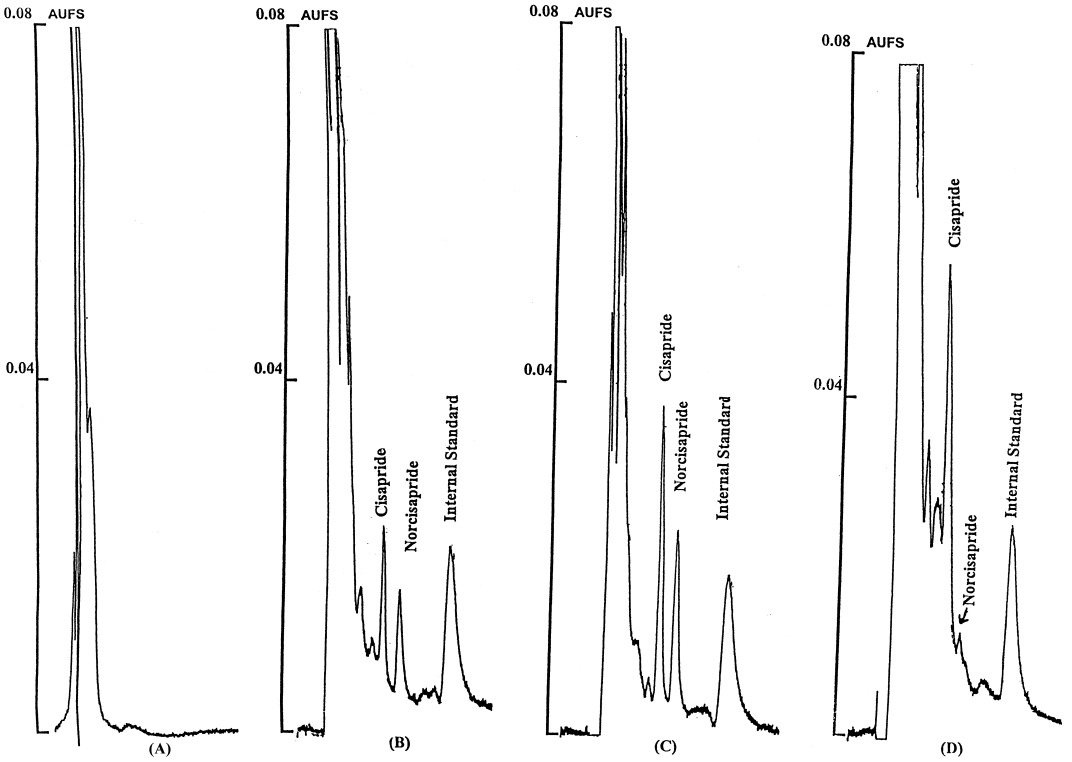


Fig. 2. Chromatogram of extracts from (A) blank control plasma, (B) control plasma spiked with 50 ng/ml of both cisapride and norcisapride, (C) control plasma spiked with 100 ng/ml of both cisapride and norcisapride and (D) plasma from a six-months-old child, after oral intake of 1.4 mg as a suspension, containing 90 ng/ml cisapride and 10 ng/ml norcisapride.

method used a large plasma volume, not well adapted to the daily analysis of numerous pediatric samples [7]. The Preechagoon method used a low plasma volume but fluorescence detection [8]. Therefore, we have tried to develop a rapid, simple and reproducible method using UV detection and quantifying both cisapride and norcisapride. Satisfactory resolution and reproducibility have been obtained for cisapride and norcisapride at concentrations ranging from 10 to 150 ng/ml. Finally, since the extraction step is simple, this method could be useful for routine determination of numerous samples.

Acknowledgements

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