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HPLC Assay for Paracetamol and Sulfapyridine in Human Plasma as Markers of Gastric Emptying and Orocecal Transit

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A simple High-performance Liquid Chromatography (HPLC) method is presented for the simultaneous determination of two marker drugs, namely paracetamol and sulfapyridine in human plasma, indirectly indicative of gastric emptying and orocecal transit, respectively. Extraction of both drugs was carried out with chloroform-isopropyl alcohol (8:2). Separation was on YMC-Packed C18 column with mobile phase comprising 1.8% tetrahydrofuran in 0.01 M sodium acetate buffer adjusted to pH 4.5, at a flow rate of 1.0 mL min^{-1} and ultraviolet detection at 254 nm. The detection limit is approximately 0.1 μg mL^{-1} for paracetamol and 0.05 μg mL^{-1} for sulfapyridine. The method is applicable to monitor gastric emptying and orocecal transit following oral administration of paracetamol and sulfasalazine, the latter being hydrolyzed in the large bowl and absorbed as sulfapyridine.

Key words: HPLC method, gastrointestinal transit, paracetamol, sulfapyridine

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INTRODUCTION

Gastric emptying has been related to the absorption of paracetamol[1], which is preferentially absorbed in the small intestine. Therefore, its overall absorption rate is determined by the rate at which the drug is moved from the stomach into the duodenum. On the other hand, sulfasalazine when administered orally is hydrolyzed by the bacterial flora of the large intestine to produce sulfapyridine and 5-aminosalicylic acid. Absorption of sulfapyridine in the plasma can be used for the determination of the orocaecal transit time[2,3].

Administration of both paracetamol and sulfasalazine will therefore enable estimation of both gastric and orocecal transit time. However, the procedure requires a specific and sensitive method for analysis of paracetamol and sulfasalazine. Individual methods have been reported for paracetamol[4] and sulfasalazine [5,6] but none was for analyzing the two drugs simultaneously. Using these drugs as the markers, it seems possible to assess the bioavailability of drug in dosage forms by monitoring their gastric emptying times and small intestinal transit time. This study reported a specific and sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous determination of the two compounds in plasma to reduce the time and cost of analysis.

MATERIALS AND METHODS

Materials: Paracetamol, Sulfapyridine, β-Hydroxyethyltheophylline were obtained from Sigma Chemical Co. (USA) and all the solvents or reagents used were either AR or HPLC grade.

Instrumentation: The HPLC system comprised a Jasco PU-980 Intelligent HPLC pump, a Gilson 119 UV/VIS detector (Gilson Medical Electronics, Villiers-le-Bel, France) connected to a Hitachi D-2500 integrator (Hitachi, Tokyo, Japan) and a Rheodyne 7125 sample injector fitted with a 50 μL sample loop. The detector was operated using a sensitivity range of 0.005 AUFS. A YMC-Pack ODS (5 μm, 150x4.6 mm internal diameter) column fitted with an Upchurch refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) packed with Perisorb RP-18, 30-40 μm pellicular stationary phase was used for the chromatographic separation. The mobile phase comprised 1.8% tetrahydrofuran in 0.01 M sodium acetate buffer adjusted to pH 4.5 with glacial acetic acid. Analysis was run at a flow rate of 1.0 mL min^{-1} with a detection wavelength of 254 nm and the peak area was used for quantification.

Standard solutions: Stock solutions of paracetamol, sulfapyridine and β-Hydroxyethyltheophylline were prepared by dissolving 100 mg of each compound with 100 mL methanol. The standard curves were prepared by spiking drug free plasma with known amounts of paracetamol and sulfapyridine at five concentration levels of each drug. The concentration levels used for paracetamol were 0.5, 1.0, 2.0, 4.0 and 8.0 μg mL^{-1}, while the concentrations used for sulfapyridine were 0.18, 0.36, 0.72, 1.44 and 2.88 μg mL^{-1}. The standard plasma samples were stored at -20°C in glass bottles.

Extraction procedure: Prior to injection, the drugs were extracted from the plasma samples according to the following procedure: 0.25 mL aliquot of plasma sample was measured accurately into an Eppendorf microcentrifuge tube, followed by the addition of 50 μL (25 μg mL^{-1}) β-hydroxyethyltheophylline (BHET) internal standard and 1.0 mL of 8:2 chloroform-isopropyl alcohol extracting solvent. The mixture was vortexed for 1 min using a vortex mixer and then centrifuged at 12,800 g for 2 min. The supernatant was transferred into a reactivial (Pierce Reacti-vial, USA) and then evaporated to dryness at 60°C under a gentle stream of nitrogen gas. After evaporation to dryness, the residue was reconstituted with 100 μL of mobile phase and 50 μL was injected into the column.

Assay validation: Samples were quantified using peak area ratio of each drug over the internal standard. Recovery, within-day and between-day precision and accuracy studies (n=6) were carried out using these plasma standards. The recovery of the extraction procedure for each drug and internal standard were calculated by comparing the peak area obtained after extraction with that of aqueous solution of corresponding concentrations without extraction. The accuracy was expressed as percentage error, obtained by calculating the percentage of difference between the measured and the spiked concentration over that of the spiked value, whereas the precision was expressed by the coefficient of variation (CV). In addition, detector linearity was determined using the standard solutions of paracetamol and sulfapyridine over a concentration range of 0.25-8.0 and 0.18-2.88 μg mL^{-1}, respectively.

RESULTS AND DISCUSSION

Chromatograms obtained with blank plasma and with plasma spiked with paracetamol, sulfapyridine and BHET are shown in Fig. 1a and 1b. The peaks obtained with three compounds are well resolved and free of interference from endogenous substances in the plasma. The blank chromatogram shows a clean baseline at retention times of 6.9 min for paracetamol and 12.7 min for sulfapyridine while 9.0 min for internal standard, BHET.

Table 1: Recovery values for paracetamol and sulfapyridine (n=6)

Paracetamol		Sulfapyridine	
Conc. ($\mu g\ mL^{-1}$)	Recovery% (CV %)	Conc. ($\mu g\ mL^{-1}$)	Recovery% (CV %)
0.5	78.8 (3.2)	0.18	88.3 (7.4)
2.0	80.2 (2.2)	0.72	87.5 (5.8)
8.0	77.6 (1.8)	2.88	86.8 (4.4)
Mean	78.9		87.5

Table 2: Precision and accuracy of assay for paracetamol and sulfapyridine (n=6)

Paracetamol			Sulfapyridine		
Spiked conc. ($\mu g\ mL^{-1}$)	CV (%)	Accuracy (%)	Spiked conc. ($\mu g\ mL^{-1}$)	CV (%)	Accuracy (%)
Within-day					
0.5	3.72	106.8	0.18	6.68	103.98
1.0	2.82	103.5	0.36	3.74	105.76
2.0	2.54	101.2	0.72	4.45	101.60
4.0	2.04	98.8	1.44	3.23	100.00
8.0	1.95	99.3	2.88	5.57	102.59
Between-day					
0.5	5.41	98.60	0.18	7.22	100.71
1.0	4.54	99.40	0.36	5.30	98.56
2.0	3.49	100.18	0.72	2.88	98.84
4.0	2.79	100.45	1.44	2.46	97.39
8.0	2.18	100.40	2.88	1.58	102.20

Internal standard is structurally related to the methylxanthines, such as caffeine and theobromine. Some of these compounds are found in food and beverages, the method should be free of interference with these substances. The tetrahydrofuran content in the mobile phase was found to be crucial in separating these compounds and it also influenced their elution order. By disturbing the ratio of the mobile phase different effects upon separation of these compounds were observed such as by increasing the ratio of tetrahydrofuran the retention time of all the compounds were decreased and the peaks were diffused instead of sharp peaks.

The recovery of paracetamol and sulfapyridine was determined by comparing the peak area obtained by direct injection of standard aqueous solutions to those obtained after the plasma extraction procedure. A mixture of chloroform and isopropyl alcohol (8:2) gave better recoveries for paracetamol and sulfapyridine as well as for internal standard compared to using n-hexane, diethyl ether, ethyl acetate, isopropyl alcohol and chloroform alone as the extracting solvent. The recovery values of the extraction procedure are presented in Table 1. The recovery of BHET was the highest averaging 93.2% followed by sulfapyridine with 87.5% and paracetamol with 78.9%. The extraction efficiency appears to be unaffected by the drug concentrations. Moreover, the CV

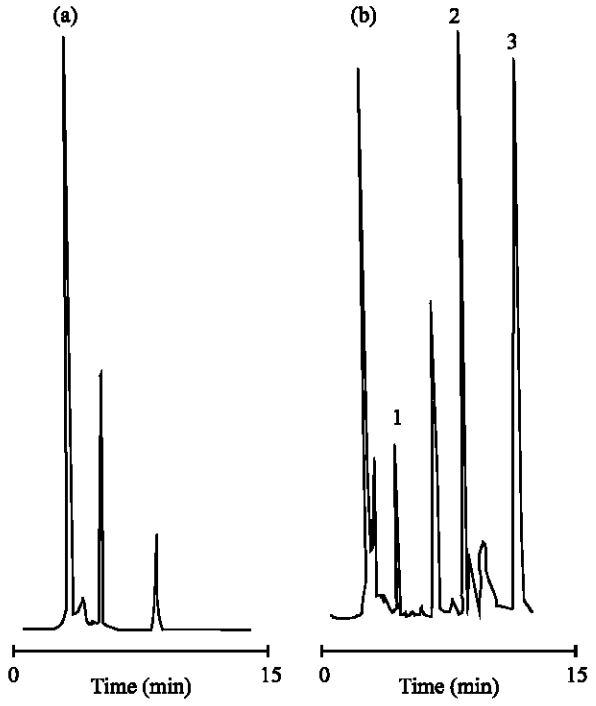

Fig. 1: Chromatograms of (a) blank plasma; (b) plasma spiked with (1) paracetamol, (2) BHET and (3) sulfapyridine

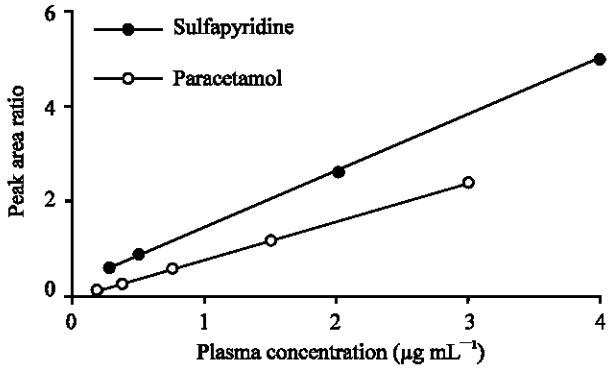

Fig. 2: Mean standard curve of paracetamol and sulfapyridine

of the recovery values for the two drugs was less than 10% at the concentrations determined.

The accuracy and precision of the method assessed by analysis of plasma samples within-day and between-day at various concentration levels, together with the recovery values of the extraction procedure are given in Table 2. The within-day and between-day CV values were relatively small for the assay of both drugs, being less than 8% at the concentration range determined. Similarly,

the within-day and between-day accuracy values also appeared to be satisfactory for both drugs at this concentration range. The limit of detection was approximately 0.1 μg mL^{-1} for paracetamol and 0.05 μg mL^{-1} for sulfapyridine at a signal-to-noise ratio of 5:1. The mean standard curve (n=6) is shown in Fig 2. A linear correlation was found between the peak ratio of paracetamol and the internal standard versus paracetamol concentration in the plasma in the range of 0.5-8.0 μg mL^{-1} with a coefficient of correlation (r) 0.9998. Similarly, linear correlation was also found between the peak ratio of sulfapyridine and the internal standard versus sulfapyridine concentration in the plasma in the range of 0.18-2.88 μg mL^{-1} with a coefficient of correlation (r) 0.9999.

From the results, it seems that the analysis time of paracetamol and sulfapyridine is relatively short, being less than 15 min and co-administration of two marker drugs (double marker) are useful in estimating the gastric emptying and the small transit time of solid dosage forms such as tablets or pellets. The most recent gamma scintigraphy technique has been used for direct monitoring of gastric emptying and intestinal transit processes[7-9]. The major advantage of double marker method is that the subjects are not exposed to any radiation and has been successfully applied in the studies of gastrointestinal monitoring of theophylline pellets[10].

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