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COMMUNICATION

A Novel Simultaneous HPLC Assay for Serum Paracetamol and Sulfapyridine as Markers of Gastric Emptying and Orocecal Transit

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ABSTRACT

A simple high-performance liquid chromatography method is described for the simultaneous determination of two marker drugs, namely paracetamol and sulfapyridine, in serum, indirectly indicative of gastric emptying and orocecal transit, respectively. Extraction of the drugs from serum was achieved with chloroform:isopropyl alcohol (7:3). Analysis was performed with a mobile phase comprising 1.9% tetrahydrofuran in 0.01 M sodium acetate buffer adjusted to pH 4.5, through a YMC-Packed C18 column at a flow rate of 1.0 ml/min and ultraviolet detection at 254 nm. The detection limit is approximately 0.2 μg/ml for paracetamol and 0.1 μg/ml for sulfapyridine. This method is applicable to monitoring gastric emptying and orocecal transit following oral administration of paracetamol and sulfasalazine, the latter being hydrolyzed in the large bowel and absorbed as sulfapyridine.

INTRODUCTION

Absorption of paracetamol has been shown to be related to the rate of gastric emptying (1). This may be attributed to paracetamol being preferentially absorbed in the small intestine. Therefore, its rate of absorption may be used as a measure of the gastric emptying rate. On the other hand, sulfasalazine, when administered orally, is hydrolyzed by the flora of the large bowel to produce sulfapyridine and 5-aminosalicylic acid. Measurement of the absorbed sulfapyridine in the blood can then be used to determine the orocecal transit time (2,3).

Administered together, paracetamol and sulfasalazine will therefore enable estimation of both the gastric emptying rate and orocecal transit time. However, the pro-

cedure requires a specific and sensitive assay method. Individual assay methods have been reported for paracetamol (4) and sulfapyridine (2,3), but none was for measuring the two compounds simultaneously. In this paper, we report a specific and sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous measurement of the two compounds in serum to reduce the time and cost of analysis.

MATERIALS

Theophylline, paracetamol, sulfapyridine, caffeine, paraxanthine, theobromine, and β-hydroxyethyltheophylline (BHET) were obtained from Sigma Chemical Co. (USA). All other chemicals and reagents used were either AR or HPLC grade.

METHOD

The HPLC system used consisted of a Jasco PU-980 solvent delivery system and a variable wavelength Jasco UV-975 ultraviolet-visible (UV-VIS) detector equipped with a Hitachi D-2500 integrator. A YMC-Pack ODS-A column (150 mm × 4.6 mm) was used for the chromatographic separation. The mobile phase comprised 1.9% 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 with the detector operating at 254 nm. Quantitation was effected using peak area.

Extraction of the drugs from serum was performed as follows: 250 μl of the serum sample was measured into an Eppendorf microcentrifuge tube, followed by adding 50 μl of β-hydroxyethyltheophylline (BHET) internal standard solution (25 μg/ml) and 1.0 ml of 7:3 chloroform-isopropyl alcohol extracting solvent. The mixture was then vortexed for 1 min and centrifuged at 14,000 rpm for 2 min. Approximately 0.9 ml of the solvent was recovered and dried under a gentle stream of nitrogen at 60°C in a reactivial. The residue was reconstituted with 100 μl of mobile phase and 20 μl injected onto the column.

Accuracy and precision studies were performed using drug-free serum spiked with both paracetamol and sulfapyridine at five concentration levels for each drug. The accuracy was calculated as a percentage of the measured concentration over the spiked value, whereas the precision was expressed by the coefficient of variation (CV). Recovery values of the extraction procedure were also determined using spiked serum samples at different concentration levels.

RESULTS AND DISCUSSION

Chromatograms obtained with blank serum, and with serum spiked with paracetamol, sulfapyridine, and BHET are shown in Figs. 1(a) and 1(b). The peaks obtained with the three compounds are well resolved and free of interference from endogenous substances in the serum. The blank chromatogram shows a clean baseline at the retention times of 7.5 min for paracetamol, 11.3 min for BHET, and 12.7 min for sulfapyridine. The internal standard, BHET, is structurally related to the methylxanthines, such as caffeine and theobromine, as well as theophylline and its metabolite paraxanthine. Since some of these compounds are found in food and beverages, the method should be free of interference with these substances. Figure 1(c) shows a chromatogram obtained from a mixture of theobromine, paracetamol, paraxanthine, theophylline, BHET, sulfapyridine, and caffeine. Satisfactory baseline separation is achieved with all the seven compounds with no interference from the above methylxanthines.

The tetrahydrofuran content in the mobile phase was found to be crucial in separating the seven compounds, and it also influenced their elution order. Other studies have also demonstrated the use of tetrahydrofuran in resolving the methylxanthines, in particular theophylline and paraxanthine (5,6).

The recovery values of the extraction procedure are tabulated in Table 1. The recovery of BHET was highest averaging 93.3%, followed by sulfapyridine with 87.2% and paracetamol with 78.7%. The extraction efficiency appears to be unaffected by the drug concentrations. Moreover, the CV of the recovery values for the three drugs was less than 10% at the concentrations determined.

The within-day and between-day accuracy and precision values of the assay method are given in Table 2. Both the within-day and between-day CV values were relatively small for the assay of both drugs, being less than 8% in all cases. The within-day and between-day

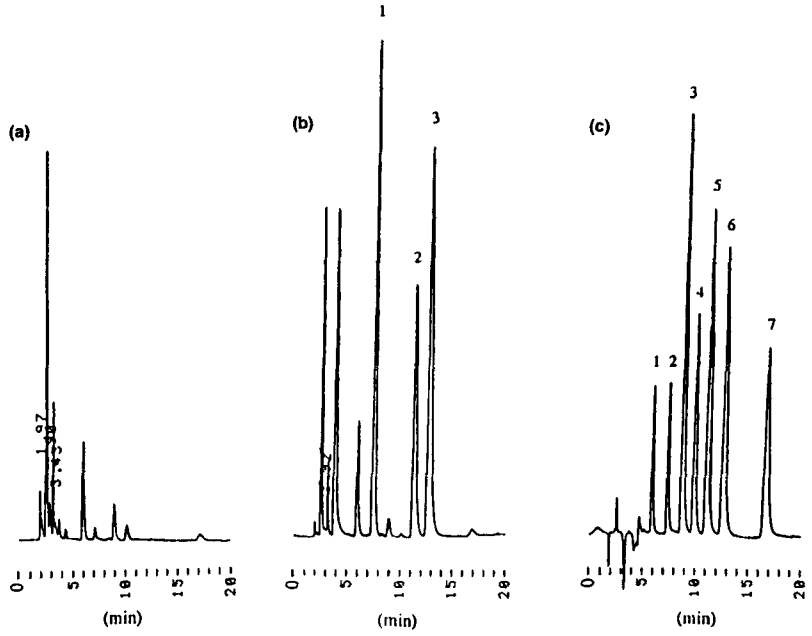

Figure 1. Chromatograms of (a) blank serum; (b) serum spiked with (1) paracetamol, (2) BHET, and (3) sulfapyridine; (c) a mixture of (1) theobromine, (2) paracetamol, (3) paraxanthine, (4) theophylline, (5) BHET, (6) sulfapyridine, and (7) caffeine.

accuracy values also appeared to be satisfactory for both drugs at the five serum concentrations determined. In all cases, the percentage error was less than ± 5%.

The detection limit of the assay method was 0.2 μg/ml for paracetamol and 0.1 μg/ml for sulfapyridine at a signal to noise ratio of 5:1, being comparable to those of the individual assay methods reported for paracetamol (4) and sulfapyridine (2,3). In addition, the detector response was also found to be linear over the range of concentrations used for both drugs.

CONCLUSION

In conclusion, the HPLC method described above is sensitive and specific for the simultaneous assay of

Table 1

Recovery Values for Paracetamol, Sulfapyridine, and BHET (n = 6)

Paracetamol		Sulfapyridine		BHET	
Conc., μg/ml	Recovery, % (CV %)	Conc., μg/ml	Recovery, % (CV %)	Conc., μg/ml	Recovery, % (CV %)
1.0	79.1 (3.1)	0.75	87.3 (8.0)	6.0	93.3 (2.7)
4.0	80.0 (1.6)	3.0	87.5 (4.8)		
16.0	77.0 (2.5)	12.0	86.7 (3.4)		
Mean	78.7		87.2		

Table 2

Precision and Accuracy of Assay for Paracetamol and Sulfapyridine (n = 6)

Paracetamol			Sulfapyridine		
Spiked Conc., μg/ml	CV, %	Accuracy, %	Spiked Conc., μg/ml	CV, %	Accuracy, %
Within-day					
1.0	3.72	104.7	0.75	5.98	103.89
2.0	2.72	99.03	1.5	2.74	104.91
4.0	2.46	99.70	3.0	6.82	101.50
8.0	1.96	98.10	6.0	3.13	102.49
16.0	1.37	103.73	12.0	4.44	100.00
Between-day					
1.0	5.13	98.40	0.75	6.74	100.50
2.0	4.64	98.20	1.5	4.20	97.29
4.0	2.59	100.45	3.0	1.08	99.80
8.0	2.69	100.40	6.0	2.36	98.59
16.0	1.17	100.16	12.0	2.48	101.00

paracetamol and sulfapyridine in serum. Both precision and accuracy errors are small over the range of concentrations determined. Moreover, analysis time is relatively short, being less than 15 min.

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