High-Pressure Liquid Chromatographic Assay of Theophylline in Dog Feces following Oral Administration of Sustained-Release Products

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Abstract □ A solid-phase-extraction reversed-phase HPLC assay is described for the determination of theophylline embedded in dog feces as powder, sustained-release tablets, or capsules. The feces is extracted with 5% isopropyl alcohol in chloroform in the presence of β-hydroxy-propyl-theophylline as the internal standard. Separation and quantitation are achieved with a C_{18} analytical column. UV absorbance is monitored at 280 nm. Recovery of theophylline was >50%. The assay is linear between 10 and 400 mg amounts of theophylline in 50 g of feces. Inter- and intraday coefficients of variation of the chromatographic assay were <3%, and the extraction procedure was highly reproducible with coefficients of variation of <10% at amounts of drug from 10 to 400 mg. By keeping the stool/solvent extraction ratio constant, the method is equally effective in extracting theophylline from different sizes of stool samples (50 versus 200 g of stool). The assay was applied to evaluate the theophylline content in feces following oral administration of the drug to dogs as tablet (Theo-Dur) and capsule (Slo-Bid) dosage forms. The resulting fecal recovery values of each product were inversely related to the corresponding bioavailability values obtained from the literature.

Theophylline, an important anti-asthmatic agent, is well absorbed at different regions of the gastrointestinal (GI) tract,[1] which make sustained-release formulations a rational and successful choice. Nonetheless, the total GI transit time (mouth-to-anus) imposes a potential limit on drug delivery from slowly releasing products, such that portions of the dose can be lost by defecation. The absorption of theophylline in dogs from several sustained-release formulations has been examined in the literature using plasma concentration–time data.[2] These results indicate that the absolute bioavailability of theophylline among these formulations (Choledyl, Theo-Dur, Theo-24, and Slo-Bid) varied considerably (63, 76, 30, and 60%, respectively).

To confirm the pharmacokinetic estimations of extent of absorption, theophylline content in feces after various dosage forms needs to be examined. No convenient assay method is available at present. More importantly, an effective extraction procedure is needed to recover the compound carried by different formulations. A sensitive and specific extraction HPLC assay has been developed in our laboratory to measure theophylline in serum, saliva, and breast milk.[3] The present communication describes our experience in modifying the approach to measure theophylline content in dog stool. The assay was used to evaluate theophylline content in feces following multiple-dose oral administration of the drug as Theo-Dur (tablet) or Slo-Bid (capsule) to dogs.

Experimental Section

Chemical Reagents—Theophylline and β-hydroxy-propyl-theophylline (internal standard) were purchased from Sigma (St. Louis, MO). Sustained-release formulations included Theo-Dur (200 mg tablets, lot #P8096, Key Pharmaceuticals, Kenilworth, NJ) and Slo-Bid (200 mg capsules, lot #78359, William H. Rorer Inc., Fort Washington, PA) and were used as obtained. Chloroform and 2-propyl alcohol were HPLC grade and purchased from Burdick and Jackson Labs (Muskegon, MI).

Chromatographic Conditions—The HPLC system consisted of a model M45 pump, a Rheodyne sample injector (model 7125), and a model 441 absorbance detector with a mercury lamp and a 280-nm filter (Water Associates, Milford, MA). Peak heights were integrated with HP3392A integrator (Hewlett-Packard, Avondale, PA). A saturator precolumn (40 mm by 4.6 mm) packed with 30–38-μm silica (Whatman, Clifton, NJ) was inserted between the pump and injector to saturate the mobile phase with silica before passing through the analytical column. Chromatographic separation was obtained on an Alltech Econosphere ODS 5 μ C_{18} column (250 by 4.6 mm). The mobile phase consisted of 9.5% (v/v) 2-propyl alcohol in sodium acetate buffer (0.82 g/L of anhydrous sodium acetate, adjusted to pH 4 with glacial acetic acid). The flow rate was 1.2 mL/min, and all chromatographic experiments were carried out at room temperature.

Extraction—Theo-Dur is formulated as a two-phase tablet consisting of pellets compressed with a matrix. Slo-Bid is in the form of long-acting beads within a hard gelatin capsule. Unevenly distributed fragments of the dosage forms in feces and variable daily fecal production by the dogs were anticipated and circumvented by analyzing daily whole stool samples.

Each stool sample was initially diluted 1:2 (w/v) with deionized water, which included 25 mL of 0.1% β-hydroxy-propyl-theophylline as internal standard. The mixture was mechanically shaken for 2 h, followed by extraction with 5% 2-propyl alcohol in chloroform for another 2 h, with mechanical shaking. Prolonging the extraction time further did not enhance the extraction efficiency. The organic solvent was added in the ratio of 4:1 (v/w) with respect to the original weight of the stool. Aliquots of the pseudo-homogenate were transferred and centrifuged at 3500 rpm for 30 min. The organic extract layer was separated and transferred to a clean test tube and evaporated under a stream of nitrogen. About 0.5 to 4 mL of deionized water was added (volume was inversely proportional to the weight of the stool), and the sample was vortexed for 1 h. A 15-μL aliquot of the final solution was injected in the HPLC column.

Analytical Tests—*Range of Linearity*—Known amounts (10 to 400 mg) of theophylline powder were added to blank dog stool samples of 50 g each. Standard curves were made by plotting the peak height ratios between theophylline and internal standard against the amount of theophylline added.

Formulations—To evaluate the capability of the procedure in extracting theophylline from various formulations, 200 mg of theophylline as intact Theo-Dur tablet, Slo-Bid capsule, or powder was added separately to 50 g of blank stool. The corresponding peak height ratios were then compared.

Stool/Solvent Ratio—A ratio of 1:2:4 (w/v/v) of stool:aqueous:organic solvent was used for extraction. To evaluate the consistency of this ratio in extracting theophylline from stool samples of different sizes, 200 mg of theophylline powder were added to 50 g and 200 g of blank stool samples. The resulting peak-height ratios of the two treatments were then compared.

Recovery—Recovery of the extraction procedure was examined by comparing the peak height ratio between the theophylline powder added before and after extraction. The amount chosen corresponded to 200 mg of theophylline in 50 g of dog stool.

Dog Theophylline Bioavailability Study—Four beagle dogs were treated orally with either 400 mg once-a-day doses of Theo-Dur

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tablets or Slo-Bid capsules for periods of 5 consecutive days in a balanced crossover fashion separated by a 3-week washout interval. Each dose was administered in the morning after an overnight fast. Blood samples were collected at frequent intervals, and stool samples were collected from the metabolic cages daily for 8 consecutive days of each study period to assess possible unabsorbed drug. Blood and stool samples were frozen at −20 °C until analysis. For the purpose of this paper, only the fecal data obtained from this study will be discussed.

Results and Discussion

Figure 1 shows the chromatogram of blank dog fecal extract spiked with internal standard, and the chromatograms of the typical fecal extract from a dog following Slo-Bid administration. No endogenous peaks were observed following the extraction step, and the theophylline peak was not contaminated by the metabolites. Potential metabolites produced by the dog differ in retention times (Table I). Several HPLC methods with C_{18} columns and acetate buffer systems have been reported in the literature for the analysis of theophylline in a variety of biological fluids.[4-7] The present study shows that this chromatographic system is also applicable in analyzing stool sample.

Amounts of theophylline recovered from the stools were determined. The chromatographic assay was linear between 10 and 400 mg, with correlation coefficients of >0.99. The amounts of theophylline back-calculated from the regression line were mostly within 10% of the expected values. Intra-day (repeated injection) and interday CVs of the HPLC assay were <3% (Table II). Reproducibility of the extraction method was quite satisfactory (Table III) as indicated by CV values that were <10% at all theophylline amounts.

A study of the ability of the method to extract theophylline from intact or broken tablets and capsules was conducted. Weighed fragments or the whole products were added to the diluted blank stool homogenates, and then the extraction procedure was conducted. Results (Figure 2) indicate that all three dosage forms (powder, tablet, capsule) at various conditions are superimposable on the same regression line. Similar peak-height ratios were observed when theophylline (200 mg) embedded in the stools was in the form of powder,

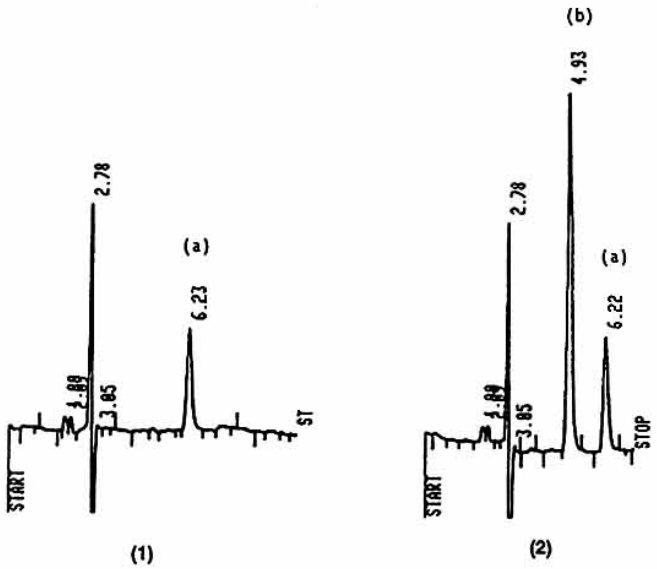

Figure 1—Chromatograms of (1) a blank dog fecal extract spiked with β-hydroxy-propyl-theophylline (a) as internal standard and (2) a typical dog fecal extract with theophylline (b) administered as Slo-Bid. Numbers on chromatograms are retention times (min) of the compounds.

Table I—Relative Retention Time of Various Compounds Related to Theophylline

Compound	Relative Retention Time[a]
β-Hydroxy-propyl-theophylline	1.23
Theophylline	1.00
1,3-Dimethyluric acid	0.71
3-Methyluric acid	0.70
1-Methyluric acid	0.64

[a] Relative to theophylline.

Table II—Accuracy and Variability of Theophylline Chromatographic Assay in Dog Stool[a]

Amount Added, mg	Intraday		Interday	
	Amount Found, mg	CV, %	Amount Found, mg	CV, %
10	9.04	2.77	9.16	1.08
100	102	0.85	101	1.96
400	411	0.22	410	0.26

[a] Number of determinations, 4.

Table III—Reproducibility of the Extraction Method for Theophylline in Fecal Samples[a]

Amount Added, mg	Amount Found, mg	CV, %
10	9.01	3.96
50	58.4	4.30
100	103	8.78
200	193	5.95
400	395	2.81

[a] Number of determinations, 4.

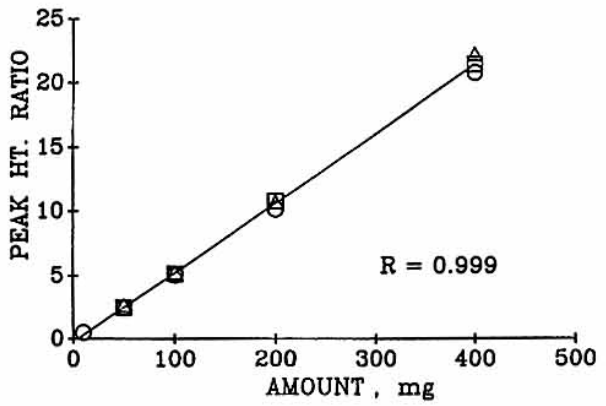

Figure 2—Relationships between peak-height ratios and the amounts of theophylline added to the blank stool homogenates in the forms of (○) powder (10, 50, 100, 200, and 400 mg), (□) Slo-Bid (1/4, 1/2, 1, and 2 capsules), and (△) Theo-Dur (1/4, 1/2, 1, and 2 tablets).

tablets, or capsules (Figure 3). By applying the same stool/solvent extraction ratio, similar peak-height ratios were obtained from 200 mg of theophylline powder embedded in 50- and 200-g stool samples (Figure 3).

The extractability of the method is limited by the ratio of aqueous and organic solvents. A large quantity of water is needed to maintain fluidity of the extraction medium and to minimize the differences in fluid contents of the stools produced on different days and from different dogs. Under the present stool/solvent composition ratio, reasonably high frac-

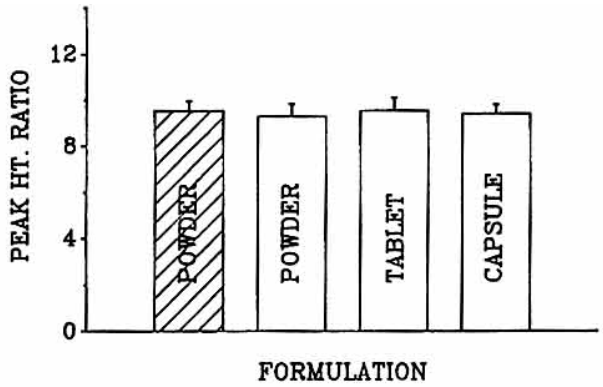

Figure 3—Comparisons of the peak-height ratios (mean ± SD, n = 4) of theophylline resulting from adding 200 mg of the drug in various forms to (□) 50 or (▨) 200 g of blank stool samples.

tions of the doses (200 mg) were extracted into the organic phase (mean, 54%; CV, 8.6%). The use of an internal standard further improved the accuracy and sensitivity of the assay. The lowest quantitative amount of theophylline was 10 mg, which is equivalent to 2.5% of a commonly prescribed 400-mg daily dose of the drug. Lesser values are expected to have an insignificant influence on the bioavailability estimations of the various products.

The method was applied to assess the stool contents of theophylline after multiple-dose Slo-Bid and Theo-Dur treatments to dogs. Figure 4 depicts the daily fecal productions and the corresponding theophylline contents at the two treatment periods from a typical animal. High fluctuations were observed in daily fecal productions of the same dog as well as among the animals (range, 11–273 g). Fluctuations were also observed in daily fecal theophylline recoveries from the same

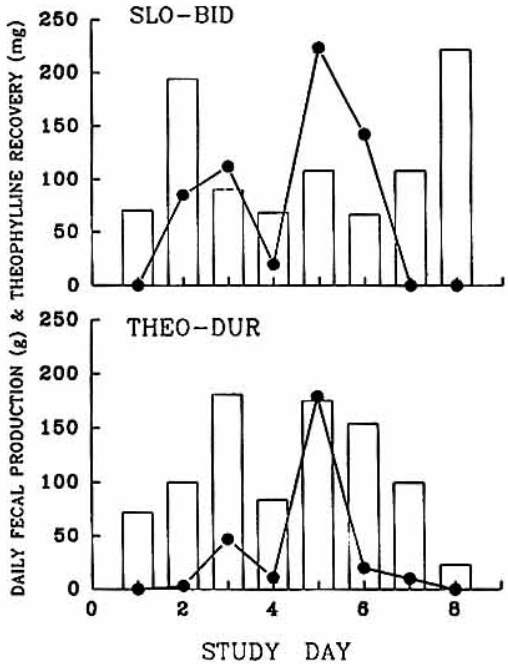

Figure 4—The daily amounts of (□) feces produced and (●) theophylline recovered from a dog during the Slo-Bid and Theo-Dur treatment periods.

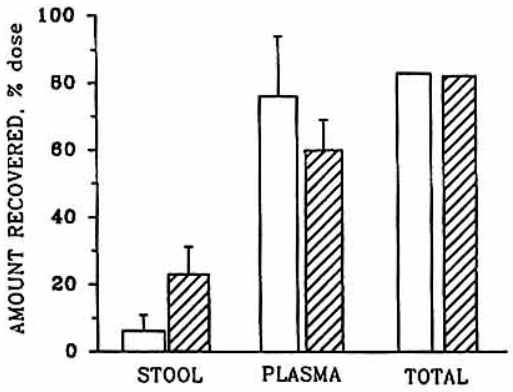

Figure 5—Comparisons of the daily average recovery of theophylline in stool (mean ± SD), plasma (mean ± SD), and stool plus plasma (total) of the dogs after (□) Theo-Dur and (▨) Slo-Bid treatments. Plasma values were obtained from the literature (ref 2).

animal as well as among the dogs (range, 0–223 mg). Figure 5 shows the dose-averaged recovery values of theophylline (% dose) in stool from the animals after administration of Theo-Dur and Slo-Bid. Values were considerably higher from Slo-Bid (mean ± SD, 23 ± 8.3% dose; range, 14–31%) than from Theo-Dur (mean ± SD, 6.2 ± 4.7% dose; range, 2.8–13%). The absolute bioavailability values of Slo-Bid (mean ± SD, 60 ± 9% of dose) and Theo-Dur (mean ± SD, 76 ± 18% of dose) in beagle dogs have been determined from plasma concentration data.[2] When the two sets of mean recovery values (stool and plasma) are combined, the total amount of theophylline recovered is essentially identical between Theo-Dur (82.2% dose) and Slo-Bid (83% dose) in the dog (Figure 5). These findings confirm the hypothesis that bioavailability of the slow-releasing product (Slo-Bid) is limited by the GI transit absorption window of the dogs (total GI transit time of dogs averages 17 h[8]).

In summary, a sensitive, specific, and reliable extraction/HPLC method for measuring theophylline in dog stool was developed. The method is equally operative in extracting the compound from three solid dosage forms. The entire extraction and chromatographic procedure can be accomplished within a reasonably short period of time. The high theophylline contents recovered in the stool justify the important contribution of assaying stool samples in bioavailability assessment of theophylline sustained-release products.

References and Notes

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Acknowledgments

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