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Determination of a novel bile acid sequestrant in rodent diet by near-infrared spectroscopy

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Abstract

DMP 504 is a high molecular weight polymer currently under development by The DuPont Merck Pharmaceutical Company as a novel bile acid sequestrant to lower serum cholesterol. To assess its safety, DMP 504 is incorporated into rodent diet for oral administration to rats and mice. An analytical method was developed to determine the accuracy and homogeneity of the blends. Since a physical separation or extraction of DMP 504 from the diet was not feasible, near-infrared spectroscopy (near-IR) was employed. The near-IR method provides accurate and precise results for blends containing 1.5–8.0% of DMP 504. Comparison of results at the 1.5% level with a cholate binding referee method is also presented. Both methods provided equivalent results for the 1.5% level.

Keywords: Bile acid sequestrant; Rodent diet; Near-infrared spectroscopy

1. Introduction

Bile acid sequestrants bind bile acids in the lower intestine, therefore increasing the level of bile salts in the feces. Since the bile salts cannot be reabsorbed, the body must synthesize new bile salts in the liver from cholesterol. Some of this cholesterol is derived from blood plasma, resulting in a net reduction in plasma levels of cholesterol [1,2]. A novel bile acid sequestrant, DMP 504, is currently being developed by The DuPont Merck Pharmaceutical Company. DMP 504 is an insoluble condensation copolymer of hexamethylene diamine and 1,10-dibromodecane (Fig. 1).

In the course of the development of pharmaceuticals, analytical methods need to be developed to measure such properties as concentration, purity and stability. For DMP 504 these measurements cannot be easily done because, to date, no solvent for DMP 504 has been identified. This lack of a solvent makes DMP 504 unsuitable for analysis by the traditional chromatographic and spectro-

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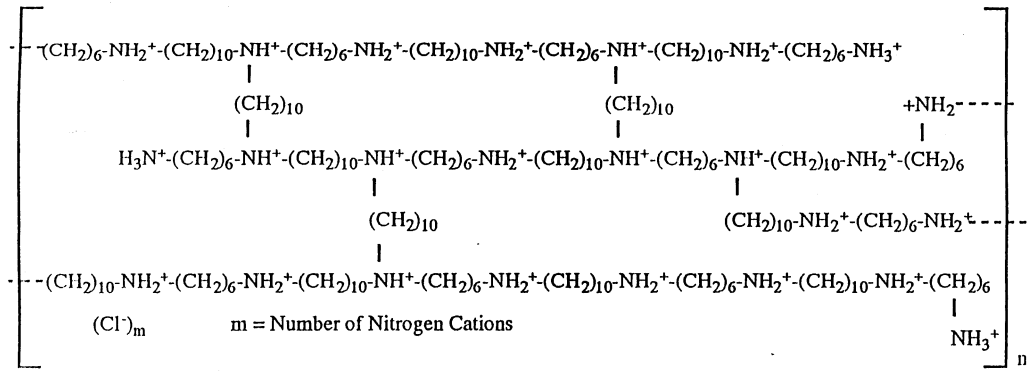

Chemical Formula: $(C_{18}H_{40}N_2Cl_2)_n$ (Approximate)

Relative Molecular Mass: 355.4

Fig. 1. The structure of DMP 504.

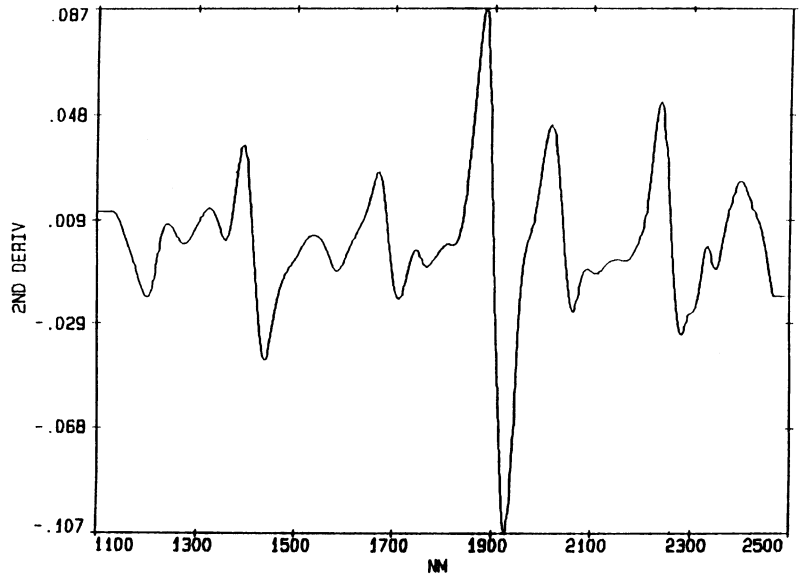

Fig. 2. The second derivative plot of the original near-IR function.

scopic techniques applied to pharmaceutical compounds. Therefore, new and specific techniques to analyze DMP 504 needed to be developed.

Near-infrared spectroscopy (near-IR) has been successfully employed in the past for pharmaceutical and agricultural analysis [3–7]. The tech-

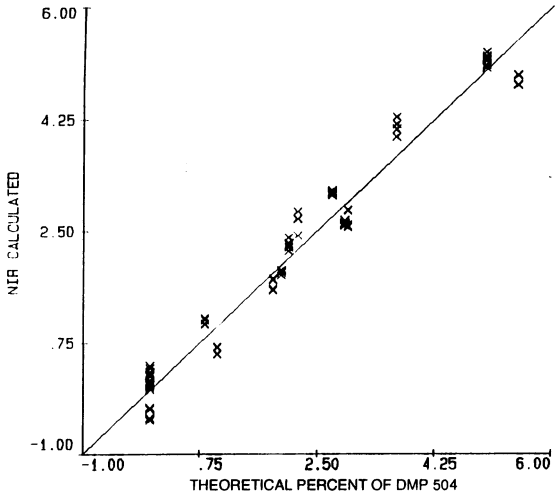

Fig. 3. Linearity plot for near-IR calculated value vs theoretical percent of DMP 504.

nique is particularly useful for cases in which sample extraction is time consuming or the matrix introduces interferences. Although a cholate binding assay has been successfully employed in the past for analysis of bile acid sequestrants [1,2,8], the near-IR technique was developed because of the inextractability of DMP 504 from the diet matrix. The absorbances exhibited by DMP 504 in the near-IR region may be exploited to provide accurate and precise determinations of the concentration in the diet blends. A near-IR calibration training set was developed using generally accepted principles based on residual variance methods [9–11]. Cross correlation with a cholate binding method at the 1.5% level shows good agreement between the methods. The near-IR method has proven to be reproducible and rugged.

Table 1
Recovery results for rodent diet fortified with DMP 504

Fortification level (wt/wt%)	% Recovery ± SD	*n*
1.0	97.0 ± 3.8	24
3.0	98.8 ± 5.7	42
5.5	100.0 ± 2.7	42
8.0	96.0 ± 3.7	24

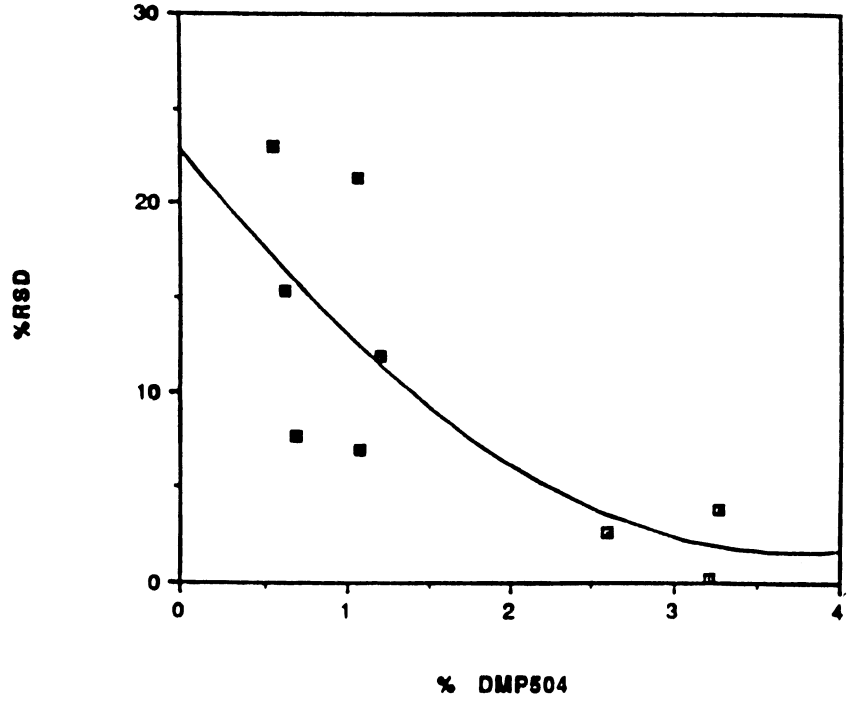

Fig. 4. Plot of % RSD vs percentage of DMP 504.

2. Experimental

2.1. Reagents and materials

The near-IR spectrophotometer used for these studies was a 6500 series with the rotating drawer attachment from Perstorp Analytical. (Silver Spring, MD). Rodent diet was PMI Rodent Laboratory Chow 5002 (Purina, St. Louis, MO). The DMP 504 drug substances were provided by The DuPont Merck Pharmaceutical Company (Wilmington, DE). The blends actually used throughout the toxicity studies were prepared by Bio-Research Laboratories, Senneville, Quebec, Canada under contract with DuPont Pharma, Mississauga, Ontario, Canada.

2.2. Procedure

A master calibration curve was derived which included 75 calibration standards incorporating several different lots of both rodent diet and active drug substance. Measurements were taken using the rotating drawer attachment operating in diffuse reflectance mode with a spectral resolution of 4 nm. In order to incorporate extremes of anticipated variability in standard blends several parameters were included in developing the calibration set. Four different lots of DMP 504 drug substance, each with at least two different levels of water content (≈ 1 and 5% water) were used. Three different lots of feed prepared from two different harvest cycles were used in the preparation of the standards. Prepared standards were stored under both constant room light and constant darkness conditions for periods between 0 and 21 days prior to incorporation into the calibration set. This calibration curve has been used repeatedly to quantitate many different preparations of blended diet samples. For each set of samples analyzed, a set of working standards was prepared using the same diet and lot of DMP 504 as the samples. Four working standard concentrations are prepared including a non-fortified blank. The working standards were prepared at concentrations that closely bracketed the expected concentrations of the samples. The working standards were analyzed by triplicate scans of duplicate samplings. The samples were analyzed by triplicate scans of triplicate samplings. Different protocols were used for the analysis of samples and standards because the 'working standards' were used to adjust the slope of the master calibration curve. An additional sampling for the samples was taken to ensure that the total sample weight used was representative of the total sample provided for analysis. Approximately 2 g of the blend was needed for each analysis.

Table 2
Values obtained for the analysis of basal diet samples

Basal diet sample number	Near-IR calculated value for % DMP 504
1	0.063
2	−0.037
3	−0.015
4	−0.184
5	−0.188
6	−0.134
7	−0.169
8	−0.029
9	0.203
10	0.315
11	0.280
12	−0.135
13	−0.183
14	−0.078
15	−0.328
16	−0.356
17	−0.358
18	0.041
19	0.100
20	−0.127

2.3. Mathematical manipulations

Each near-IR spectrum collected was converted to the second derivative of the original function using a segment size of 10 and a gap size of zero (Fig. 2). The wavelengths used for quantitation were 1723 and 1634 nm. The absorbance at 1723 nm is characteristic of DMP 504. The absorbance at 1634 nm is characteristic of the feed. This combination provided a good correlation and maximum sensitivity. The DMP 504 concentration in each calibration standard (expressed as a percentage) was plotted vs the ratio of the values

Table 3
Study sample results

Preparation		Study A		Study B		
		% of Target ± SD				
		1.5% Level	3.0% Level	2.0% Level	3.0% Level	5.0% Level
1	Top	109.4 ± 5.1	103.3 ± 2.5	96.9 ± 5.4	99.4 ± 2.3	101.2 ± 5.8
	Middle	106.7 ± 2.0	103.1 ± 5.7	94.2 ± 7.8	103.0 ± 1.3	97.8 ± 2.4
	Bottom	104.4 ± 0.7	105.1 ± 3.3	93.9 ± 11.6	92.9 ± 0.2	102.5 ± 0.6
2	Top	107.8 ± 1.1	103.8 ± 3.2	131.0 ± 6.4	122.3 ± 2.8	109.7 ± 3.1
	Middle	107.4 ± 0.7	103.6 ± 1.4	126.7 ± 7.7	121.8 ± 5.0	114.3 ± 2.1
	Bottom	108.1 ± 0.7	109.3 ± 8.7	129.5 ± 9.3	121.4 ± 2.3	112.9 ± 2.3
3	Top	112.0 ± 2.5	94.3 ± 3.9	103.5 ± 2.4	100.4 ± 3.3	102.5 ± 2.2
	Middle	108.8 ± 1.6	102.1 ± 2.8	99.4 ± 2.1	97.2 ± 4.7	102.0 ± 2.4
	Bottom	105.9 ± 2.3	103.4 ± 2.3	103.8 ± 3.1	95.9 ± 1.3	100.0 ± 4.2
4	Top	104.7 ± 1.2	105.1 ± 7.9	109.8 ± 5.3	101.9 ± 1.1	102.1 ± 2.7
	Middle	102.5 ± 3.4	106.3 ± 2.3	108.4 ± 0.6	104.4 ± 3.5	104.4 ± 1.2
	Bottom	108.5 ± 0.8	99.7 ± 4.4	107.0 ± 2.8	103.9 ± 2.8	102.8 ± 2.3

of the second derivative at 1723:1634 nm. This provided the near-IR calculated value for each calibration standard. The calibration curve was derived by regression analysis of the near-IR calculated value derived above vs theoretical percent DMP 504. Working standards were then used to adjust the intercept of the calibration curve. The adjusted calibration curve was then used to quantitate the percent of DMP 504 in each sample.

3. Results and discussion

3.1. Linearity

The linearity of the near-IR system was evaluated over the range of 0 to 5.5% DMP 504. The system was found to be linear over the range with a correlation coefficient of 0.987 (Fig. 3).

3.2. Accuracy

Accuracy was evaluated by measuring recovery of DMP 504 in rodent diet fortified at levels of 1.0, 3.0, 5.5 and 8.0%. Recoveries ranged from 96.0 to 100.0% with standard deviations of 2.7 to 5.7 (Table 1). Recoveries were determined using the following equation:

$$A/B \times 100 \quad (1)$$

where $A = \%$ DMP 504 found by near-IR and $B =$ Theoretical % DMP 504 as prepared by the analyst.

3.3. Limits of quantitation and detection

The limit of quantitation was determined by plotting the relative standard deviation (RSD) vs percent DMP 504 (Fig. 4). The RSDs were calculated from the results of the nine measurements per sample (i.e. triplicate scans of triplicate samples). A preset limit of ± 20% RSD was considered to be the maximum acceptable variation allowable for precise quantitation. From the graph, the limit of quantitation was interpolated to be approximately 0.5%. The limit of detection was estimated from the analysis of basal diet samples containing 0% DMP 504 (Table 2). Results predicted using the calibration curve for these samples suggested a limit of detection of approximately 0.35%. This was shown empirically by analysis of samples prepared at 0.30 and 0.40%

Table 4
Results of statistical analysis at the 1.5% level

% of Target	Cholate			Near-IR		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Sample A						
Top	103.7	111.2	113.4	117.2	118.4	116.9
Middle	104.3	108.0	107.7	105.7	81.7	107.3
Bottom	103.9	104.1	105.2	98.3	100.2	112.1
Sample B						
Top	107.2	107.1	109.1	109.8	104.3	105.4
Middle	108.2	107.0	106.9	99.8	108.6	118.3
Bottom	108.8	108.0	107.5	104.8	108.6	102.8
Sample C						
Top	112.8	114.1	109.2	116.3	97.2	112.6
Middle	110.6	108.0	107.8	94.6	111.3	107.9
Bottom	109.3	105.5	102.8	106.4	100.6	110.9
Sample D						
Top	103.5	105.8	104.7	87.3	104.4	98.3
Middle	104.6	104.3	98.6	104.8	117.7	109.7
Bottom	109.1	107.6	108.8	99.9	104.6	94.5
Mean ± SD						
Sample A	106.8 ± 1.17			106.4 ± 3.94		
Sample B	107.8 ± 0.27			106.9 ± 1.77		
Sample C	108.9 ± 1.16			106.4 ± 2.47		
Sample D	105.2 ± 1.06			102.4 ± 2.29		

DMP 504. These samples provided a signal-to-noise ratio of approximately 3.2 whereas a ratio of 3.0 is generally considered to be the limit of detection of an analytical method.

3.4. Study sample results

Results for four different formulations of two different studies are provided in Table 3. Samples were taken from the top, middle and bottom of each blend for confirmation of accuracy and homogeneity. The near-IR results ranged from 92.9 to 131.0% of target. The probability that the difference found would exist if the true difference between locations were 0 for 1.5 to 5.0% DMP 504 blends ranged from 0.10 to 0.81. This indicates that there was no significant statistical difference among top, middle and bottom samplings and therefore, the blends were considered homogeneous.

3.5. Statistical analysis at the 1.5% level

Results of the statistical analysis of the data at the 1.5% level are provided in Table 4 and shown graphically in Fig. 5. The analysis was done to determine if there was any statistical difference between results obtained by near-IR and those obtained by cholate binding. Top, middle and bottom samplings of four different formulations were tested by each method. A 95% symmetric Westlake interval was used to test for equivalence of the two methods [12]. In addition, an F-test was used to test for equal variances between the two methods [12]. The Westlake interval was 4.1%, which was within the acceptable limit of

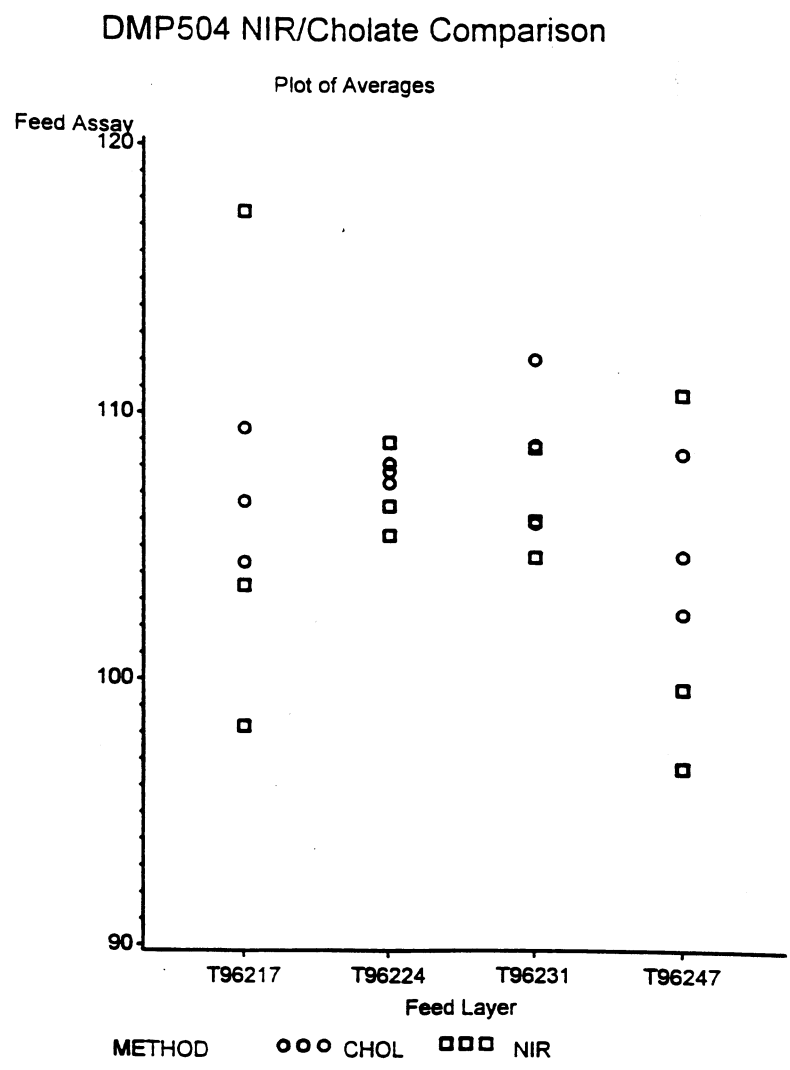

Fig. 5. Comparison of results for near-IR and cholate binding methods at the 1.5% level.

5.0%. The SDs for near-IR and cholate binding pooled across sample and location were 8.8 and 2.9%, respectively. The statistical analysis shows that the methods provide equivalent results and that the precision for cholate binding is better at the 1.5% level.

4. Conclusions

A near-IR method has been developed for the analysis of DMP 504 in rodent diet. This method can provide accurate and precise results for samples from 1.0 to 8.0% DMP 504. The accuracy of results obtained using an historical calibration curve prove that the standards included in the curve are representative of the actual samples being tested. The limits of quantitation and detection for near-IR were determined to be approximately 0.5 and 0.35%, respectively. Comparison with a cholate binding referee method at the 1.5% level showed good agreement between the two methods. Cholate binding provides better precision at or below 1.5% and will be the method used

for those samples. Above 1.5%, the near-IR method is the overall method of choice because it provides quick analyses with minimal sample preparation while the cholate binding method is more labor intensive.

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