ACKNOWLEDGMENTS AND ADDRESSES

Received December 17, 1973, from the *Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, TN 37203, and the ‡ Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow 3, India.

Accepted for publication March 4, 1974.

Supported by U.S. Public Health Service NIH Grants ES00782, ES00267, and DA00141 and by the Council of Scientific and Industrial Research, New Delhi, India.

The authors thank Professor K. P. Bhargava and Professor Stanley J. Brumleve for their advice and encouragement. Grateful acknowledgment is made to Dr. M. L. Dhar and Dr. Nitya Anand of the Central Drug Research Institute, Lucknow, India, for providing microanalysis facilities and to the Council of Scientific and Industrial Research, New Delhi, India, for providing a Senior Research Fellowship to B. Ali.

x To whom inquiries should be directed. Present address: Department of Physiology and Pharmacology, School of Medicine, University of North Dakota, Grand Forks, ND 58201

PHARMACEUTICAL ANALYSIS

Determination of Silicon in Streptomycin by Atomic Absorption

R. J. HURTUBISE

Abstract □ An atomic absorption method was developed for determining silicon in streptomycin without prior separation. The method has a reproducibility of ±2.2 ppm and a detection limit of 5 ppm silicon. Ash-fused and untreated samples were compared, and a deuterium arc background corrector indicated a moderately high bias in the results.

Keyphrases □ Silicon—atomic absorption determination in streptomycin □ Streptomycin—atomic absorption determination of silicon content □ Atomic absorption spectroscopy—determination of silicon in streptomycin

Because the manufacturing of pharmaceuticals involves many complex chemical and physical processes, contaminants can be introduced in many places before the final product is obtained. Thus, there is a need for precise, accurate, and sensitive analytical methods to determine contaminants. Silicon can be introduced by raw materials, water, and other sources into many in-process manufacturing samples. In the pharmaceutical industry, the control of the amount of silicon is important because it can directly or indirectly cause turbidity in solutions of product samples.

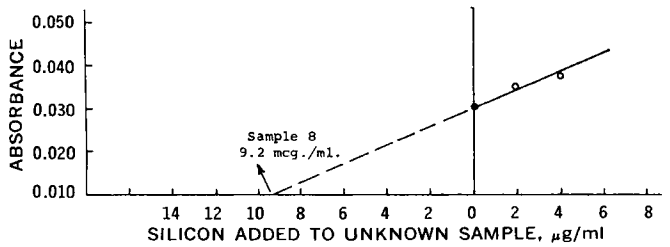

Figure 1—*Typical standard addition curve for streptomycin.*

Few atomic absorption methods have been published for the determination of silicon in pharmaceuticals (1, 2). This paper describes a rapid, sensitive, and accurate atomic absorption method for the determination of silicon in the antibiotic, streptomycin. Ashing and alkaline fusion steps are not needed, and a deuterium arc background corrector may be employed, depending on the accuracy desired.

EXPERIMENTAL

Apparatus—An atomic absorption spectrophotometer[1] equipped with a nitrous oxide burner and a deuterium arc background corrector[2] were used according to the manufacturer's instructions. A single-element silicon hollow-cathode lamp[3] was used for all silicon determinations. The following instrument settings were used for all measurements: resonance line, 251.6 nm; slit 4; lamp current, 40 mamp; nitrous oxide flow, 13 liters/min; and acetylene flow, 12 liters/min. Experimental results indicated that Pyrex volumetric glassware was suitable for this work.

Reagents—Fisher certified sodium silicate atomic absorption standard was used to prepare all silicon standards.

Procedures—*Determination of Silicon*—Between 24.0 and 26.0 g of streptomycin sulfate was weighed accurately into a 50-ml volumetric flask and diluted to volume with distilled water. Ten-milliliter aliquots of this solution were transferred quantitatively to three 25-ml volumetric flasks. Aliquots (1 and 2 ml) of 50 μg/ml standard sodium silicate solution were added to two of the volumetric flasks, respectively, and the contents of all three flasks were diluted to volume with distilled water. The atomic absorption unit was adjusted so there was a fuel-rich flame, the absorbance was set at zero with distilled water, and the absorbance of the three solutions was determined. A standard addition curve was prepared by plotting absorbance against micrograms of silicon per milliliter added to streptomycin solutions. The line inter-

[1] Perkin-Elmer model 403.
[2] Perkin-Elmer.
[3] P-E Intensitron lamp.

Table I—Recovery of Silicon

Sample	Silicon[a] Present, ppm	Silicon Added, ppm	Silicon[b] Found, ppm	Silicon Added, ppm	Silicon[b] Found, ppm	Silicon Added, ppm	Silicon[b] Found, ppm
1	47	9.5	9.0	19	19	47	47
2	70	10.0	7.8	20	17	52	42
3	65	9.8	7.4	20	15	49	37
4	66	9.8	7.4	20	15	49	37
5	70	10.0	8.0	20	17	50	42
6	65	9.9	9.9	20	20	50	48
7	54	10.0	10.0	20	20	50	47
8	46	9.9	8.5	20	18	50	47
9	55	10.0	8.4	20	17	50	47
10	56	9.9	8.4	20	17	49	46
11	74	10.0	8.0	20	15	50	40
12	54	9.6	9.6	19	17	48	45
Average		9.9	8.5	20	17	50	44
Average difference		1.4 ppm		3.0 ppm		6.0 ppm	
Average recovery, %		86		85		88	

[a] Results obtained from standard addition method. [b] Results corrected for original silicon content present in the sample.

secting the absorbance axis was extrapolated to zero absorbance to obtain the concentration of silicon in the 25-ml unknown solution and then the parts per million of silicon in the streptomycin was calculated (Fig. 1).

Determination of Silicon with Fusing—A 5.0-g sample of streptomycin sulfate was weighed accurately into a platinum crucible and then carefully ashed over a burner until all carbon was removed. One gram of sodium bicarbonate was added to the carbon-free sample and heated on a hot plate for approximately 10 min; then the sample was heated over a Meker burner until a clear or slightly turbid melt was obtained. The sample was cooled and 11.5 ml of 1 *N* H_2SO_4 was added slowly with stirring. The sample was then heated for approximately 10 min on a hot plate. The sulfuric acid solution was transferred quantitatively from the crucible to a 25-ml volumetric flask and diluted to volume with distilled water. Five-milliliter aliquots of streptomycin solution were added to three 10-ml volumetric flasks. To two of the volumetric flasks were added 1 and 2 ml of a 20 μg/ml standard sodium silicate solution, respectively. The contents of all three flasks were then diluted to volume with distilled water. The absorbance readings and the concentrations of silicon were obtained as described under *Determination of Silicon*, except the atomic absorption spectrophotometer was set at zero absorbance with a water solution that contained the same amount of sodium bicarbonate and sulfuric acid as the unknown solution.

RESULTS AND DISCUSSION

Table I indicates the accuracy of the method and gives the average percentage recovery and average difference for silicon added as sodium silicate to 12 different streptomycin samples which originally contained different amounts of silicon. The values of "silicon found, ppm" were obtained as follows. The unknown absorbance was subtracted from the absorbance of the corresponding spiked samples; these absorbance difference values were used to obtain the corresponding concentrations of silicon from a calibration curve prepared from water solutions of sodium silicate; finally the parts per million of silicon found was calculated. The average percentage recovery was low in all cases, and the average difference between the "added" and "found" silicon showed a proportional increase with increasing amounts of silicon. These effects are most likely due to the complex composition of streptomycin resulting in both chemical and physical interferences in the nitrous oxide–acetylene flame. To minimize these interferences, the standard addition method was used. Since the unknown and standards were submitted to the same experimental conditions, the same proportional decrease in absorbance was manifested in both, so proportional errors were eliminated.

Table II gives the average absorbance and standard deviation corresponding to the samples in Table I. Slight composition variation of the different samples was not considered important. The results indicate that as the concentration of silicon increases, the absorbance values become less reliable. Because of the poorer reliability at high silicon concentrations, it was decided to use only two standard additions per determination.

The precision of the method (Table III) was obtained from replicate determinations on five different samples of streptomycin. If the results from Sample 14 are omitted, an average reproducibility of ±2.2 ppm silicon can be expected. The poorest precision for Sample 14, which contained about twice as much silicon as the other samples, was probably due to incomplete dissociation of silicon compounds in the nitrous oxide–acetylene flame. The results in Table I support this because, as the amount of silicon increases, the difference between the added silicon and the experimental silicon increases.

Since the standard addition method only minimizes proportional errors, sample size was varied to detect constant errors. The results in Table IV show that variation in sample size does not indicate any large constant errors.

Table II—Precision of Absorbance Values[a]

	2 μg Silicon/ml Added	4 μg Silicon/ml Added	10 μg Silicon/ml Added
Average absorbance	0.004	0.008	0.020
Standard deviation	0.000	0.001	0.002

[a] Absorbance values corrected for original silicon content present in samples.

Table III—Precision of Standard Addition Method

Sample	Silicon[a], Average ± ppm	Number of Determinations
8	47 ± 4.3	6
12	56 ± 2.8	6
13	46 ± 0.97	24
14	105 ± 12	6
15	52 ± 0.56	6

[a] Determined at the 95% confidence level.

Table IV—Variation of Sample Size[a]

Sample Weight, g	Silicon, ppm
11.025	52
16.797	50
22.260	52
26.043	49
31.520	48
	$\overline{50}$ ± 2.2 ppm[b]

[a] Sample 13. [b] Precision determined at 95% confidence level.

Table V—Comparison of Ashed-Fused and Untreated Samples

Sample	Silicon (Ashed-Fused), ppm	Silicon (Untreated), ppm	Difference, ppm Silicon
12	64	56	+8
16	51	46	+5
17	60	53	+7
18	41	51	−10
19	58	53	+5
20	27	36	−9
21	30	41	−11
22	36	45	−9
23	41	44	−3
24	51	61	−10
		Average	−2.7

Because silicon can exist in many forms such as silicates, soluble silica, and colloidal silica (3), the silicon content was determined in both ashed-alkaline fused samples and untreated samples to decide if total silicon was determined by the proposed standard addition method. The results (Table V) show a random distribution of difference values, indicating that indeterminate errors are present. Because the proposed standard addition method has an average reproducibility of ±2.2 ppm silicon, the indeterminate errors most likely result from the ashing and fusing steps. Application of the statistical t test at the 95% confidence level indicated that there was no significant difference between the results from ashed-fused samples and untreated samples. Thus, total silicon was determined in streptomycin by the proposed standard addition method.

Because the standard addition method does not eliminate background absorption such as molecular absorption and absorption due to the solvents or salts aspirated into the flame (4), a deuterium arc background corrector was used to see if effects of this type were present. Aqueous silicon standards gave identical readings with and without background absorption. The results in Table VI indicate that without using the deuterium arc background corrector the results for streptomycin samples were biased high by approximately 6 ppm silicon. Consequently, to obtain more accurate results, a deuterium arc corrector should be used. However, the error introduced was not large and, depending on the accuracy needed, the use of the deuterium arc corrector could be omitted. No attempt was made to identify the source of this error, but ions such as calcium, iron, and sodium can enhance the response of silicon (5). These ions are present at trace levels in streptomycin.

Table VI—Comparison of Results with and without Deuterium Arc Background Corrector

Sample	Silicon[a], ppm		Difference	Number of Determinations
	Arc	No Arc		
25	58 ± 3.7	63 ± 3.0	−5	6
26	80 ± 2.4	86 ± 2.4	−6	6

[a] Precision determined at the 95% confidence level.

To determine the limit of detection, it was necessary to have silicon-free samples of streptomycin. Because such samples were not readily available, the limit of detection was calculated as the concentration of silicon that would give an absorbance of 0.002. Since a sample with 50 ppm silicon would have an absorbance of 0.020, a sample with 5 ppm would give an absorbance of 0.002. Thus, the limit of detection is near 5 ppm silicon.

REFERENCES

(1) E. Mario and R. E. Gerner, *J. Pharm. Sci.*, **57**, 1243(1968).

(2) J. R. Miller, J. J. Helprin, and J. S. Finlayson, *ibid.*, **58**, 455(1969).

(3) M. J. Taras, A. E. Greenberg, R. D. Hoak, and M. C. Rand, "Standard Methods for the Examination of Water and Wastewater," 13th ed., American Public Health Association, Washington, D.C., 1971, p. 300.

(4) H. L. Kahn, *Atomic Absorption Newsletter*, **7**, 40(1968).

(5) W. J. Price, "Analytical Atomic Absorption Spectrometry," Heyden and Son, New York, N.Y., 1972, p. 143.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 29, 1973, from *Quality Control, Pfizer Inc., Terre Haute, IN 47808*

Accepted for publication February 22, 1974.

The author acknowledges the technical assistance of E. W. Fagg in this work and J. A. Means for his helpful comments on this manuscript.

New Method for Quantitative Determination of D-Dopa in Levodopa Samples[x]

A. VIDI, C. FRANCO, and G. BONARDI

Abstract □ A simple method for the determination of D-dopa present in levodopa samples is reported. The method is based on the property of the L-amino acid oxidase to convert quantitatively levodopa to 3,4-dihydroxyphenylpyruvic acid while D-dopa remains unchanged. The latter is assayed fluorometrically, and contaminant amounts of D-dopa present in levodopa samples can be detected.

Keyphrases □ Levodopa—quantitative determination of D-dopa contaminant □ D-Dopa—quantitative determination in levodopa samples □ Fluorometry—analysis, D-dopa in levodopa samples

The analytical assay of the purity of levodopa preparations, used in the treatment of Parkinson's disease, allows the detection of the D-enantiomer which may cause undesirable side effects (1, 2).

Previous work (3) suggested a method for determining minimal amounts of D-dopa present in levo-