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.

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

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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-

Table I—Determination of D-Dopa in Levodopa Standard Preparations

Standard Preparations	D-Dopa Found[a] mg	%
Levodopa, 10.000 mg, + D-dopa, 0.500 mg	0.492	98.32
Levodopa, 10.000 mg, + D-dopa, 0.200 mg	0.195	97.42
Levodopa, 10.000 mg, + D-dopa, 0.100 mg	0.095	95.60
Levodopa, 10.000 mg, + D-dopa, 0.050 mg	0.048	96.66
Levodopa, 10.000 mg, + D-dopa, 0.025 mg	0.027	103.02
	Mean ± *SD*	98.20 ± 1.28
Levodopa, 10.000 mg	0.000	0.000
D-Dopa, 10.000 mg	9.998	99.980

[a] Values represent average of three determinations.

dopa samples. This method is based on the property of the L-amino acid oxidase (EC 1.4.3.2) enzyme to convert selectively and quantitatively levodopa into 3,4-dihydroxyphenylpyruvic acid. According to Anton and Sayre (4), the latter does not interfere in the subsequent fluorometric assay of the unchanged D-dopa.

EXPERIMENTAL

In a test tube (26 × 110 mm) fitted with a cap and equipped with tubes for the bubbling of oxygen (1 bubble/sec), 10.0 mg of standard levodopa[1], various amounts of standard D-dopa[1], 10.0 mg of pure L-amino acid oxidase[1], 30.0 μg of flavine-adenine dinucleotide[1] in a total volume of 10.0 ml of 0.1 *M* phosphate buffer (pH 6.5) were mixed. The mixture was incubated at 37° for 3 hr in the dark and subsequently centrifuged at 40,000×*g* for 20 min at 4°. The supernate was diluted 1:200 with 0.1 *M* phosphate buffer (pH 6.5), and D-dopa was determined fluorometrically[2] according to Anton and Sayre (4). The excitation and emission wavelengths were 330 and 380 nm (uncorrected data), respectively.

[1] Sigma.

[2] A model MPF-2A spectrophotofluorometer (Hitachi, Ltd.) was used.

RESULTS AND DISCUSSION

From the results reported in Table I, the validity of the method is confirmed by incubating separately pure samples of levodopa and D-dopa with amino acid oxidase enzyme. Levodopa is converted to 3,4-dihydroxyphenylpyruvic acid quantitatively while D-dopa is recovered unchanged. The dilution of the supernate is performed to obtain minimal interferences and D-dopa concentrations lower than 2 μg/ml where the response to the fluorometric assay is linear. The sensitivity limit for the determination of D-dopa in the presence of levodopa is 0.25%.

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Determination of Disodium Edetate Dihydrate in Streptomycin by Atomic Absorption Spectrophotometry

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Abstract □ An atomic absorption method was developed for determining disodium edetate dihydrate (I) in the antibiotic streptomycin. It is free of phosphate interference and has a reproducibility of ±1.3 μg/g and a limit of detection near 4 μg/g. Quantitation involves the formation of a nickel–I complex, release of the complexed nickel by pH adjustment, and the determination of nickel by atomic absorption. The amount of nickel determined is directly proportional to the amount of I.

Keyphrases □ Streptomycin—determination of disodium edetate dihydrate, atomic absorption spectroscopy □ Disodium edetate dihydrate—determination in streptomycin, atomic absorption spectroscopy □ Edetate disodium dihydrate—determination in streptomycin, atomic absorption spectroscopy

In the production of pharmaceuticals, small amounts of disodium edetate dihydrate (I) may be used to remove alkaline earth impurities and to improve the efficiency of production processes. To control low levels of I in pharmaceuticals, rapid, accurate, and precise analytical methods are important. A search of the chemical literature yielded one method for I in pharmaceuticals (1). Other methods have been developed for I in waste water, urine, and protein solutions (2–6). No methodology was reported