Table IV—Determination of Dipyrone in Injections Containing Variable Amounts of Sodium Sulfite by the USSR Pharmacopoeia IX Method

Sample Number	Content of Dipyrone, g.	Content of Sodium Sulfite, mg.	Recovery, %
1	0.5010	00.0	100.81
2	0.5020	14.0	105.73
3	0.5030	23.0	112.67
4	0.5030	38.0	118.11
5	0.5010	48.0	126.79

nitroso derivative of one of its hydrolysis products (27). Scheme I illustrates the suggested mechanism for the formation of such a derivative.

REFERENCES

(1) N. Buric, *Farm. Glas.*, **17**, 109(1961).

(2) "State Pharmacopoeia of the Union of Soviet Socialist Republics," IX.

(3) M. E. Shub and N. A. Kobzareva, *Aptechn. Delo*, **5**, 48 (1956).

(4) G. Milch, *Gyogyszereszet*, **4**, 342(1960).

(5) M. P. Wirth, *Pharm. Acta Helv.*, **32**, 383(1957).

(6) T. Gulubova and M. Iliev, *Farmatsiya*, **15**, 116(1965).

(7) R. Vasieliv, V. Scintee, I. Chialda, E. Sisman, J. Fruchter, and M. Jecu, *Rev. Chim. (Bucharest)*, **13**, 759(1962).

(8) J. Baltazar and M. M. Ferreira Barga, *Rev. Port. Farm.*, **14**, 116(1964).

(9) E. Schulek and L. Maros, *Acta Pharm. Hung.*, **27**, 237(1957).

(10) K. Kigasawa, H. Shimizo, S. Watari, N. Ikari, and H. Mikubo, *Yakugaku Zasshi*, **84**, 638(1964).

(11) O. Kalejs and M.E. Volkova, *Aptechn. Delo*, **9**, 45(1960).

(12) J. Fecko, *Acta Polon. Pharm.*, **20**, 225(1963).

(13) A. Y. Ibadov, Z. N. Nazirov, and T. Dreventsova, *Tashkentsk. Farm. Inst.*, **3**, 326(1962).

(14) T. Tao and H.-Y. Yu, *Yao Hsueh Hsueh Pao*, **8**, 206(1960).

(15) T. Murata and T. Ochiai, *Kumamoto Pharm. Bull.*, **4**, 15(1959).

(16) F. Maggiorelli and L. Conti, *Farmaco, Ed. Prat.*, **15**, 179 (1960).

(17) S. Ono, R. Onichi, and K. Kawamura, *Yakugaku Zasshi*, **85**, 239(1965).

(18) K. Kawamura, *ibid.*, **87**, 394(1967).

(19) I. Kolchev and I. Bencev, *Farmatsiya*, **10**, 11(1960).

(20) K. Kato, M. Umeda, and S. Tsubota, *Yakuzaigaku*, **24**, 116(1964).

(21) H. Matsumoto, *Arch. Pract. Pharm.*, **25**, 304(1965).

(22) N. P. Yavorskii, V. S. Koval, and M. M. Starushchenko, *Farm. Zh. (Kiev)*, **20**, 31(1965).

(23) D. Drygieniec and J. Balkowska, *Farm. Pol.*, **22**, 753(1966).

(24) G. Guadagini and G. Sekulcs, *Boll. Chim. Farm.*, **103**, 438 (1964).

(25) C. Silveira and M. T. Barrosa, *Rev. Port. Farm.*, **14**, 96 (1964).

(26) O. Naobumi, *Yakugaku Zasshi*, **85**, 1001(1965).

(27) I. M. Roushdi, S. A. Soliman, and M. H. Sadek, *UAR J. Pharm. Sci.*, **13**, 347(1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 21, 1973, from the *Pharmaceutical Analytical Chemistry Department, College of Pharmacy, University of Alexandria, Alexandria, Egypt.*

Accepted for publication June 29, 1973.

* Present address: The Alexandria Co. for Pharmaceuticals, Alexandria, Egypt.

▲ To whom inquiries should be directed.

Optimum Conditions for GLC Analysis of Neomycin

MICHEL MARGOSIS*▲ and KIYOSHI TSUJI†

Abstract □ A previously described GLC method for the analysis of neomycin was critically examined to delineate the problems involved and to resolve the difficulties encountered in the procedure. The breakdown and/or adsorption of the silyl derivatives is minimized by modifying the injection port of one instrument so as to eliminate all metal and Teflon contact with the injected material.

Keyphrases □ Neomycin—GLC analysis, optimum conditions □ GLC—analysis, neomycin, optimum conditions

Neomycin is an antibiotic used extensively throughout the world. Many dermal, renal, and ototoxic reactions associated with its therapeutic use have been reported in the literature. These reports, however, do not indicate the chemical composition of the antibiotic bulk used in commercial formulations, although neomycin is known to be comprised of at least five different components in variable amounts (1). These neomycin fractions have different responses to various microorganisms and may also differ in therapeutic activity and potency as well as in pharmacological properties. Improved methods of analysis are necessary to elucidate and characterize the chemical nature of these substances.

A reported GLC method (2), which is regularly utilized at The Upjohn Company, appears to be the best, practical method available for the qualitative and quantitative analysis of neomycin fractions, particularly neomycin B and neomycin C isomers which normally predominate. The method had been under evaluation at the National Center for Antibiotic Analysis (NCAA) for some time. Test results by The Upjohn and NCAA laboratories were comparable for the potency values of commercial bulk samples containing a fairly low amount (less than about 5%) of neomycin C. However, there was poor interlaboratory correlation between results for the neomycin C content for most bulk samples. Be-

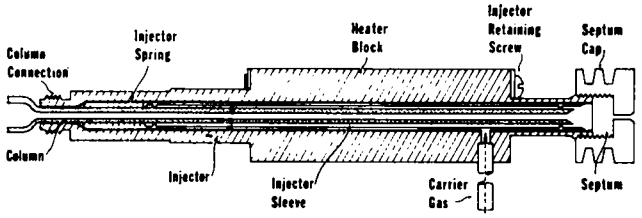

Figure 1—*Modification of injector block and column.*

cause of this discrepancy and other difficulties encountered in the performance of the analysis, a step-by-step critical examination of the procedure was undertaken by the two laboratories in close collaboration. This first resulted in two modifications of the procedure (3), namely: (*a*) use of a 0.61- or 0.91-m. (2- or 3-ft.) column packed with 3% OV-1 on Gas Chrom Q, 100–120 mesh, instead of a 1.83-m. (6-ft.) column with 0.75% OV-1 on Gas Chrom Q; and (*b*) combination of the trilaurin internal standard and the trimethylsilyldiethylamine reagent directly into Tri-Sil Z.

The objective of this article is to discuss the problems associated with this analysis.

EXPERIMENTAL

When the analyses were initially performed at NCAA[1], there were indications of serious sample degradation and solvent (pyridine) tailing. The direct on-column injections with a 15.24-cm. (6-in.) needle, as suggested by the manufacturer, did not alleviate the problem and proved too cumbersome for continuous repetitive application. To counteract these difficulties, the gas chromatograph was modified by drilling out the abutting metal separator ring in the injector block which connects the column to the injector system through a Teflon ferule and a Swagelok. A glass tube, about 14 cm. (5.5 in.) × 4 mm. o.d., was fused to one end of the column, extending it to the septum (Fig. 1)[2].

This column was packed within 1 cm. of the septum and plugged, if desired, with a minimum amount of silanized glass wool, thus minimizing any dead space. Glass wool is not really necessary in the inlet but is customarily used as a plug; it has at times been found to be deleterious to the analysis of silyl derivatives[3]. Caution is necessary when extending this column so that it fits easily into the metal sleeve of the injector block and that no undue strain, which could result in breakage, is applied when tightening the Swageloks.

This system provides a direct on-column injection, eliminates the glass insert, and obviates precolumn contact of sample to metal or glass at such elevated temperatures as 300°, as in this case. Another beneficial side effect is that solvent tailing is materially decreased.

RESULTS

Standard solution mixtures containing neomycin B (USP Reference Standard, Issue 1) and from 0 to 40% (w/w) of neomycin C [8246-DNR-58, 90.5% pure, with no neomycin B present as determined by GLC (4)] and several commercial samples were carefully prepared and analyzed. Since equal detector response was found for each neomycin isomer when used singly, the neomycin C content in all cases was calculated as the fraction of the sum of both peak areas.

Recovery of neomycin C was *not* linear at NCAA, even though the same packing material was used at both laboratories. However, results were reproducible and could be correlated with standard calibration mixtures for quantitative purposes. Further investigations revealed that the incomplete recovery of the neomycin C fractions was actually due to adsorption of the silylated derivatives onto the Teflon disk of the injector seal assembly in the gas chromatograph. Upon removal of this Teflon disk, linearity of recovery of the neomycin C fraction was achieved on an absolute scale, thus eliminating the need for calibrating standard mixtures which are not easily available. Concurrent analysis of several commercial lots in the laboratories of The Upjohn Co. and at NCAA have confirmed the usefulness of the method. The neomycin C values obtained by the triangulation method with the modified gas chromatograph at NCAA correlated well with those obtained with the electronic integrator attached to the other gas chromatograph at Upjohn. A paired *t*-test of the two sets of results listed in Table I showed no statistical difference at the 95% confidence level (calculated $t = 0.67$ for $n = 19$).

Table I—Determination of Neomycin C in Prepared Standard Mixtures and Commercial Bulk Neomycin Sulfate by GLC

Sample	Neomycin C, %: Lab 1[a]	Lab 2[b]
Standard 1	0.0	0.0
Standard 2	3.88	4.65
Standard 3	7.48	8.0
Standard 4	15.2	14.6
Standard 5	24.4	25.9
Standard 6	39.3	38.25
Lot 1	8.1	7.35
Lot 2	8.2	12.6
Lot 3	17.8	16.6
Lot 4	6.1	6.25
Lot 5	10.3	9.7
Lot 6	15.9	14.2
Lot 7	5.4	5.7
Lot 8	12.3	13.2
Lot 9	8.5	8.8
Lot 10	9.4	8.2
Lot 11	6.4	8.8
Lot 12	11.3	11.5
Lot 13	6.1	5.9
Lot 14	12.0	11.83

[a] Percent neomycin C for standards is actual percent weight in weight ratio. Commercial lots were analyzed with a Hewlett-Packard 402 with electronic integrator 3370A. [b] Results were calculated from a modified Perkin-Elmer 900 by triangulation.

Table II—Troubleshooting in GLC Analysis of Neomycin

Problem	Solution
Lot-to-lot variations in moisture content and solubility	Freeze dry and cap promptly
Indication of sample decomposition	Eliminate all metal contacts; ensure a complete glass system
Darkening of packing material in inlet	Use longer silylation time; use minimum amount of silanized glass wool and replace as needed
Asymmetrical neomycin peak	Readjust amount of trimethylsilyldiethylamine
Stability of trimethylsilyl derivative of neomycin	Use sealed reaction vials; store at refrigerated temperatures
Lack of quantitation	Use on-column injections; remove all metal and/or Teflon in inlet system; inject immediately after withdrawing sample from vial; maintain about same amount of sample injected to reproduce peak size and to improve statistics
On-column adsorption of trimethylsilyl derivative of neomycin	Remove Teflon from inlet; condition with trimethylsilyl derivative of neomycin
Short column life	Reduce column temperature (<300°); compensate with increased carrier flow rate

[1] On a Perkin-Elmer 900 gas chromatograph.
[2] These modified columns are now available custom made from Applied Science Labs., Inc., State College, Pa.
[3] W. Supina, Bellefonte, Pa., personal communication, 1971.

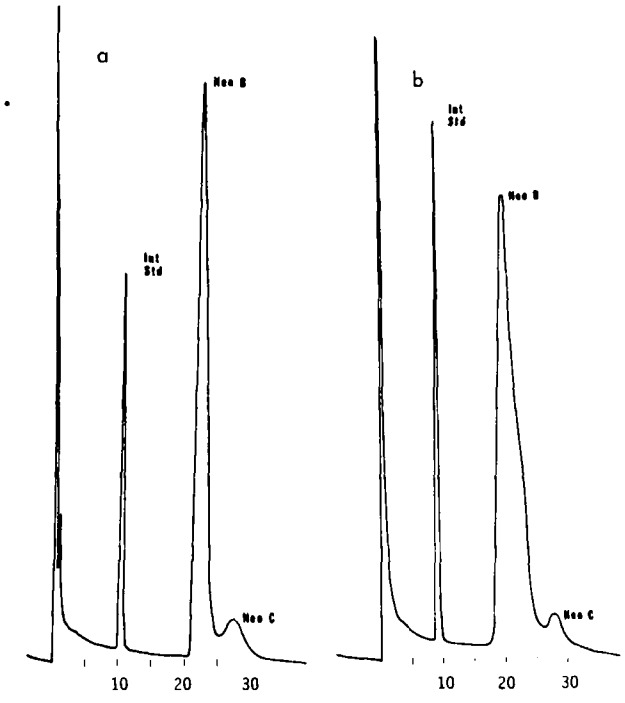

Figure 2—*Chromatogram of trimethylsilyl derivative of neomycin with: (a) proper amount of trimethylsilyldiethylamine, and (b) excess trimethylsilyldiethylamine.*

DISCUSSION

Because of the many potential sources of difficulties, strict adherence to the reported procedure (2, 3) is recommended to obtain reliable results. These problems are enumerated in Table II for convenience; and although each one is of material significance, the following are emphasized:

1. The column must be properly sample conditioned immediately before actual analysis.
2. The amount of trimethylsilyldiethylamine reagent added is critical in the formation of the derivative, as are the temperature and duration of the reaction (3). When the derivative is properly prepared, the neomycin B peak is symmetrical (Fig. 2).
3. Samples should be chromatographed as soon as possible after derivatization or kept refrigerated minimally until time of analysis so as to retain optimal stability. These neomycin derivatives degrade readily (3).

In summary, with proper techniques and caution, the GLC method for analysis of the neomycins can be successfully employed. This method has already been shown to be more specific and reliable than the microbiological method (4, 5). Even with GLC instruments offering poor linearity in recovery, satisfactory results can be obtained when they are correlated with authentic or reference standard calibration mixtures approximating the concentration level to be determined. This type of discussion might prove beneficial in similar analyses of high molecular weight oligosaccharides.

REFERENCES

(1) E. J. Hessler, H. K. Jahnke, J. H. Robertson, K. Tsuji, K. L. Rinehart, and W. T. Shier, *J. Antibiot.*, **23**, 464(1970).

(2) K. Tsuji and J. H. Robertson, *Anal. Chem.*, **41**, 1332(1969).

(3) B. Van Giessen and K. Tsuji, *J. Pharm. Sci.*, **60**, 1068(1971).

(4) J. H. Robertson, R. Baas, R. L. Yeager, and K. Tsuji, *Appl. Microbiol.*, **22**, 1164(1971).

(5) K. Tsuji, J. H. Robertson, R. Baas, and D. J. McInnis, *ibid.*, **18**, 396(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 22, 1972, from the **National Center for Antibiotic Analysis, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, DC 20204*, and †*Control Analytical Research and Development, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication July 9, 1973.

Technical assistance of T. L. Branch and B. Van Giessen of The Upjohn Co. is acknowledged.

▲ To whom inquiries should be directed.

Determination of Copper and Manganese in Vitamin-Mineral Tablets by Atomic Absorption Spectrophotometry

YOUNG SOON CHAE, JAMES P. VACIK, and WILLIAM H. SHELVER▲

Abstract □ Atomic absorption spectrophotometry was utilized in the determination of copper and manganese in five different combination multiple-vitamin mineral tablets. The analysis of each tablet by direct determination was compared with analysis by the method of additions, and some small but statistically significant differences were noted. No molecular absorption was found in the analysis of manganese utilizing the nonresonance line of lead. The direct analysis of manganese and copper was simple and precise.

Keyphrases □ Copper—determination in multiple-vitamin mineral tablets by atomic absorption spectrophotometry □ Manganese—determination in multiple-vitamin-mineral tablets by atomic absorption spectrophotometry □ Atomic absorption spectrophotometry—determination of copper and manganese in vitamin-mineral tablets □ Vitamin-mineral tablets—determination of copper and manganese by atomic absorption spectrophotometry □ Minerals, in multiple-vitamin tablets—determination of copper and manganese by atomic absorption spectrophotometry

Because of their complex nature, multiple-vitamin mineral tablets present a formidable problem in analytical chemistry. The analysis of specific metals in the presence of numerous other minerals and a complicated organic matrix requires either complex separation schemes or an extremely specific analytical method to