It has been shown that the fluorometric method is the method of choice to assay isoproterenol formulations, since this procedure is based only on the intact isoproterenol and other decomposition products, even if present in significant amounts, do not influence the final fluorescence. The described fluorometric method has the potential for automation and thus can be of immense value to the pharmaceutical industry to monitor routinely the stability of isoproterenol formulations.

REFERENCES

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 353.

(2) L. H. Welsh and O. R. Sammul, *J. Ass. Offic. Anal. Chem.*, **51**, 176(1968).

(3) T. Higuchi and L. C. Schroeter, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 535(1959).

(4) H. Hellberg, *Sv. Farm. Tidskr.*, **64**, 493(1960).

(5) V. K. Prasad, R. A. Ricci, B. C. Nunning, and A. P. Granatek, *J. Pharm. Sci.*, **62**, 1130(1973).

(6) C. C. Chang, *Int. J. Neuropharmacol.*, **3**, 643(1964).

(7) R. Laverty and K. M. Taylor, *Anal. Biochem.*, **22**, 269(1968).

(8) K. K. Kaistha, *J. Pharm. Sci.*, **59**, 241(1970).

(9) R. L. Tse and M. J. Oesterling, *Clin. Chim. Acta*, **4**, 307(1959); M. J. Oesterling and R. L. Tse, *Fed. Proc.*, **18**, 296(1958); *Amer. J. Med. Technol.*, **27**, 112(1961).

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▲ To whom inquiries should be directed.

Head-Space GLC Determination of Triethylamine in Pharmaceuticals

MARK A. LITCHMAN▲ and RONALD P. UPTON

Abstract □ A head-space GLC procedure is described for the quantitative determination of residual triethylamine in streptomycin sulfate and methacycline hydrochloride. This method is both rapid and accurate.

Keyphrases □ Streptomycin sulfate—head-space GLC analysis for residual triethylamine □ Methacycline hydrochloride—head-space GLC analysis for residual triethylamine □ Triethylamine residue in streptomycin sulfate and methacycline hydrochloride—head-space GLC analysis □ GLC, head-space—analysis, residual triethylamine in streptomycin sulfate and methacycline hydrochloride

Triethylamine has been determined qualitatively by GC (1–3). Umbreit *et al.* (4) decomposed aliphatic amine hydrochloride mixtures in the injection port of a gas chromatograph. They then analyzed 17–170 p.p.m. of each amine in the mixture. Pesez and Bartos (5) used derivatization and colorimetry to determine triethylamine at the 15-mcg. level. Kertes (6) used colorimetry to measure triethylamine at the 5% level. Derivative formation and TLC were employed by Baudot (7) at the 20-mcg. level. None of these methods involved the determination of triethylamine in pharmaceuticals.

Table I—Preparation of Standard Curve for Determination of Triethylamine in Streptomycin Sulfate

Stock Solution, ml.	1 *M* Sodium Hydroxide, ml.	Triethylamine per Serum Vial, mcg.
1.0	9.0	1090
3.0	7.0	3270
5.0	5.0	5450

Table II—Preparation of Standard Curve for Determination of Triethylamine in Methacycline Hydrochloride

Stock Solution, ml.	1 *M* Sodium Hydroxide, ml.	Triethylamine per Serum Vial, mcg.
0.5	9.5	535
1.0	9.0	1090
2.0	8.0	2180

This report describes a simple, rapid method for determining triethylamine in streptomycin sulfate[1] and in methacycline hydrochloride [4-(dimethylamino)-1,4,4*a*,5,5*a*,6,11,12*a* - octahydro - 3,5,10,12,12*a* - pentahydroxy - 6 - methylene - 1,11 - dioxo - 2 - naphthacenecarboxamide hydrochloride][1] to levels as low as 0.05%. The principle on which this method is based is that the head-space gas over a solution of a nonvolatile solute in a sealed container will contain amounts of any volatile substances present in the solution after equilibration at a constant temperature. The quantity of solvent vapors in the head-space gas will be proportional to the concentration of the solvents in the solution.

The method of Bassette *et al.* (8) was modified for this determination.

EXPERIMENTAL

Preparation of Standard Curve—Dilute 150 μl. triethylamine[2] to 100 ml. with 1 *M* sodium hydroxide solution. Transfer 1 ml. of this

[1] Pfizer Inc., New York, N. Y.

[2] Technical grade, Matheson, Coleman and Bell, East Rutherford, N. J.

Table III—Recovery of Spikes of Triethylamine in Streptomycin Sulfate

Sample Number	Added, mcg.	Recovered, mcg.	Recovery, %
1	1090	1028	94.3
2	3270	3214	98.3
3	5450	5150	94.5

stock solution to a No. 20 serum vial[3] and add 9 ml. of 1 *M* sodium hydroxide solution. Immediately cap the vial with a rubber septum[4] and metal closure[4] and swirl. Place in a water bath at 60° for 1 hr.[5] and withdraw 1 ml. of the head-space gas using a gastight syringe[6]. Immediately inject into the gas chromatograph and record the elution curve[7]. Repeat with the other standards. The preparations of standard solutions for determining the other points of the standard

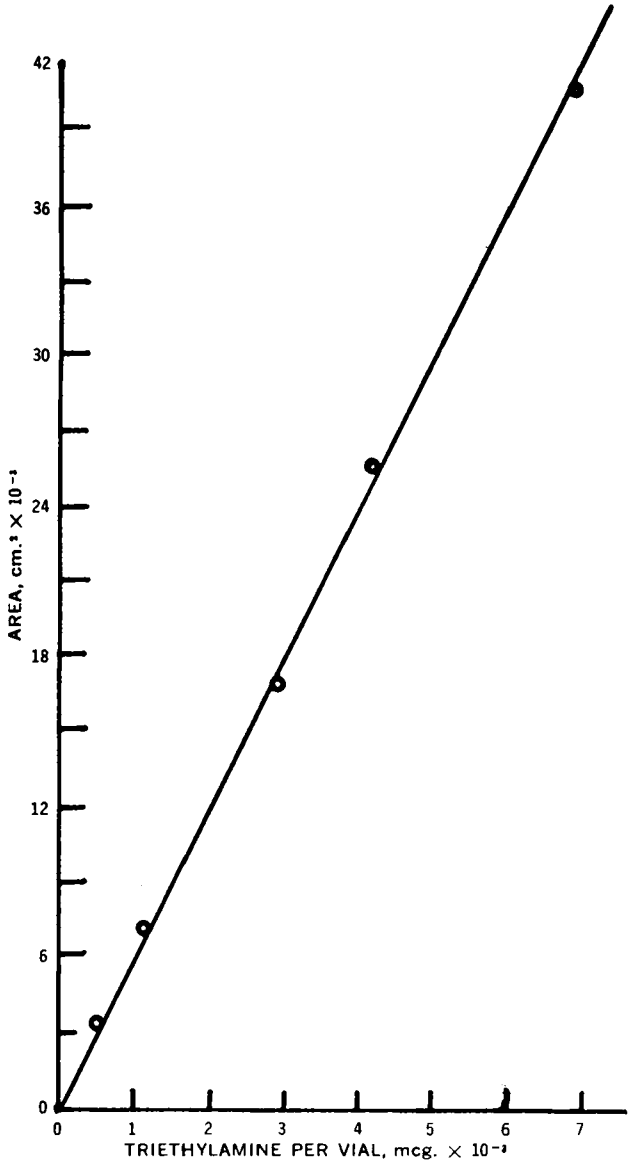

Figure 1—*Typical triethylamine standard curve.*

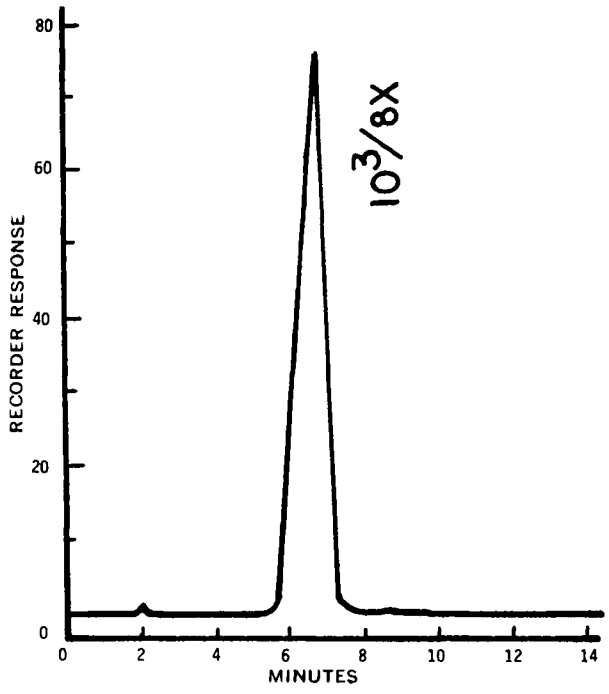

Figure 2—*Chromatogram of triethylamine in a production sample of streptomycin sulfate.*

curves for streptomycin sulfate and methacycline hydrochloride are given in Tables I and II, respectively.

Preparation of Samples—Transfer an accurately weighed 1.0-g. sample of streptomycin sulfate or 0.3-g. sample of methacycline hydrochloride to a No. 20 serum vial. Add 10 ml. of 1 *M* sodium hydroxide solution. Proceed as in *Preparation of Standard Curve*.

Gas Chromatograph—A chromatograph[8] with a hydrogen-flame ionization detector and recorder[9] was used with an electronic integrator[10] and teletype[11].

Column and Conditions—A 1.82-m. (6-ft.) stainless steel column, 0.32-cm. (0.125-in.) i.d. and 0.63-cm. (0.25-in.) o.d., was packed with polystyrene[12], 80–100 mesh, using a vibrator and conditioned at 240° with nitrogen flowing through the column for 18 hr. The operating conditions were column temperature, 160°; injector

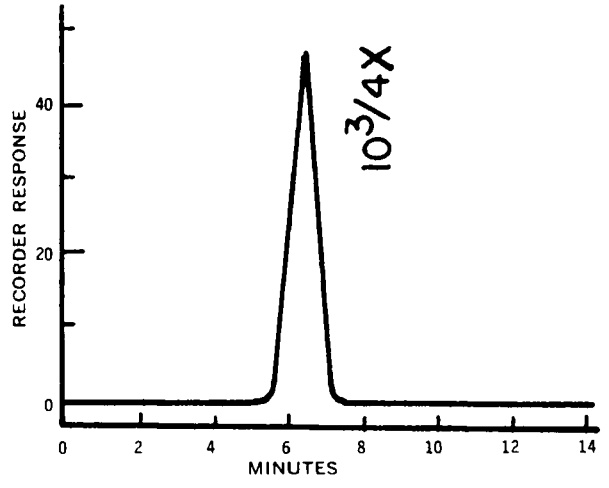

Figure 3—*Chromatogram of triethylamine in a production sample of methacycline hydrochloride.*

[3] Wheaton vial, Grand Island Biological Co., Grand Island, N. Y.
[4] Grand Island Biological Co., Grand Island, N. Y.
[5] One hour was chosen for convenience of sampling. The recovery values for triethylamine in streptomycin sulfate or methacycline hydrochloride do not change from 15 min. to 2 hr. at 60°.
[6] Precision Sampling Corp., Baton Rouge, La.
[7] The retention time of triethylamine is 6.5 min.
[8] Hewlett-Packard 5751B.
[9] Model 7127A equipped with 1-mv. span, Hewlett-Packard Corp., Avondale, Pa.
[10] Model CRS-101, Infotronics Corp., Houston, Tex.
[11] Model 33, Teletype Corp., Framingham, Mass.
[12] Porapak PS, Waters Associates., Inc., Framingham, Mass.

Table IV—Recovery of Spikes of Triethylamine in Methacycline Hydrochloride

Sample Number	Added, mcg.	Recovered, mcg.	Recovery, %
1	1090	1207	111
2	2180	2202	101
3	4360	4055	93

Table V—Determination of Triethylamine Content of Replicate Weights of a Streptomycin Sulfate Sample

Sample Number	Triethylamine Content, %
1	0.354
2	0.352
3	0.371
4	0.367
5	0.365

temperature, 235°; detector temperature, 250°; carrier gas, nitrogen, 35 ml./min.; detector gas, hydrogen, 40 ml./min.; and air, 500 ml./min.

Calculations—The area of the triethylamine peak in the samples is compared to a standard curve[13] of micrograms triethylamine per vial *versus* area found. The following formula is then used:

$$\frac{\text{micrograms triethylamine from standard curve}}{\text{weight of sample, mcg.}} \times 100 = \text{percent triethylamine} \quad \text{(Eq. 1)}$$

RESULTS AND DISCUSSION

The levels of triethylamine determined were from 0.15 to 0.36% for production samples of streptomycin sulfate, and they were from 0.06 to 0.13% for production samples of methacycline hydrochloride.

The recoveries of spikes of triethylamine in streptomycin sulfate and methacycline hydrochloride are given in Tables III and IV, respectively. The values ranged from 94.3 to 98.3% for streptomycin sulfate and from 93.0 to 111% for methacycline hydrochloride.

Table VI—Determination of Triethylamine Content of Replicate Weights of a Methacycline Hydrochloride Sample

Sample Number	Triethylamine Content, %
1	0.117
2	0.119
3	0.125
4	0.123
5	0.120

Five replicate weighings of a sample of streptomycin sulfate and methacycline hydrochloride were made, and the triethylamine content was determined (Tables V and VI). The data indicate a precision of 2 and 5%, respectively.

Examples of chromatograms for samples of streptomycin sulfate and methacycline hydrochloride are shown in Figs. 2 and 3, respectively.

The head-space method has been found to be a simple, direct, and precise method for trace volatiles component analysis. This method is currently being applied to other compounds.

[13] An example of a typical triethylamine standard curve is given in Fig. 1.

REFERENCES

(1) J. R. L. Smith and D. J. Waddington, *J. Chromatogr.*, **42,** 183(1969).

(2) S. Häntzsch, *Talanta*, **13,** 1297(1966).

(3) C. Landault and G. Guiochon, *J. Chromatogr.*, **13,** 327(1964).

(4) G. R. Umbreit, R. E. Nygren, and A. J. Testa, *ibid.*, **43,** 25 (1969).

(5) M. Pesez and J. Bartos, *Ann. Pharm. Fr.*, **23,** 781(1965).

(6) S. Kertes, *Anal. Chim. Acta*, **15,** 73(1956).

(7) P. Baudot, *J. Chromatogr.*, **59,** 203(1971).

(8) R. Bassette, S. Özeris, and C. H. Whitnah, *Anal. Chem.*, **34,** 1540(1962).

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▲ To whom inquiries should be directed. Present address: Pfizer Inc., Lee's Summit, MO 64063