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Short Communication

Determination of benzylamine in bethanidine by gas chromatography–mass spectrometry

M. G. QUAGLIA*[1], P. MAZZEO[2] and F. SECCO[3]

[1] *Istituto di Chimica Farmaceutica e Tossicologica dell'Università, Piazzale Aldo Moro 5, 00185 Roma, Italy*
[2] *Dipartimento Farmaco-Chimico dell'Università, Via Amendola 173, 70126 Bari, Italy*
[3] *Istituto di Chimica Farmaceutica e Tossicologica dell'Università, Piazzale Aldo Moro 5, 00185 Roma, Italy*

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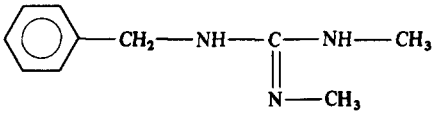
Introduction

Bethanidine (I) is widely used in the treatment of hypertension. It may contain benzylamine as an impurity, and in view of the carcinogenic nature of benzylamine



(probably arising from its transformation into a nitrosamine) and the prolonged periods of bethanidine therapy used in practice, the development of a rapid and satisfactory method of benzylamine determination in this matrix is desirable. Benzylamine analysis has been performed by gas chromatography (GLC) of trimethylsilyl [1, 2] or 2,6-dinitro-4-trifluoromethylbenzenesulphonic acid [3] derivatives, and by HPLC [4] of the *p*-nitrobenzoyl derivative. Recently [5] a direct gas chromatographic determination of amines (including benzylamine) without prior derivatization has been described. The present paper describes a rapid, accurate and precise procedure for the direct determination of benzylamine in bethanidine. Separation by GLC on an appropriate column in the presence of ammonia is followed by determination using a nitrogen–phosphorus detector (NPD) or mass spectrometry (MS).

* To whom correspondence should be addressed.

Experimental

Reagents and chemicals

Benzylamine was purchased from Merck (Darmstadt, FRG) and used without further purification. All other reagents and solvents were analytical reagent grade.

Apparatus and conditions

A Carlo Erba 2200 gas chromatograph was equipped with an NPD 40 detector (hydrogen 0.7 kg/cm^2; air 1.2 kg/cm^2). The carrier gas was helium (1.2 atm). A 120 cm × 3 mm glass column was packed with 28% Pennwalt 223 and 4% KOH on Gas-Chrom R (80–100 mesh). The temperatures used were: injection port, 250°C; column, 190°C; detector, 300°C. Mass spectrometric analyses were recorded on a low resolution mass spectrometer with a data system (LKB Model 2091/2130). The ionization energy and accelerating voltage were 20 eV and 3500 V, respectively. The ion source temperature was 250°C.

Preparation of standard solutions

The standard solutions were prepared by dilution of an ethanolic solution containing 1 ml of benzylamine and 2 ml of 25% ammonia in 100 ml, with a mixture of ethanol–25% ammonia, 25:1 v/v. Benzylamine concentrations between 1.6 and 0.08 mg/ml were studied. Calibration curves were constructed by plotting the peak areas in the gas chromatograms and in the mass fragmentograms against concentration.

Analysis of bethanidine samples

One gram of bethanidine sample was extracted at room temperature under magnetic stirring with ethyl ether for 6–8 hr. The ethereal solution was dried (Na_2SO_4) and evaporated to dryness at room pressure and temperature. The residue was dissolved in 1 ml of an ethanol–25% ammonia (25:1, v/v) mixture, and 1 μl was injected into the gas chromatograph. The fragmentographic analysis was achieved by single ion monitoring at m/z = 106, corresponding to the (M-1) ion of benzylamine. The magnet was focussed using m/z = 44 (CO_2).

Results and Discussion

Figure 1 shows the gas chromatograms of standard solutions of benzylamine in ethanol with and without ammonia. The increase in the peak areas in the presence of ammonia was probably due to the reduction of adsorption of the amine on to the glass surfaces and the column packing. The calibration curves obtained by gas chromatographic or mass fragmentographic analysis were linear over the range assayed, with correlation coefficients not less than 0.99. The minimum concentration of benzylamine detectable was 100 ppm using gas–liquid chromatography and 20 ppm using gas chromatography–mass fragmentography.

The method was tested using samples prepared by adding known amounts of benzylamine to bethanidine purified by several recrystallizations from methanol–isopropyl ether. The results are shown in Table 1. The relative standard deviation of these results was *ca* 5%. When the procedure described was applied to the analysis of four commercial bethanidine samples, benzylamine levels of 240, 340, 120 and 180 ppm were found.

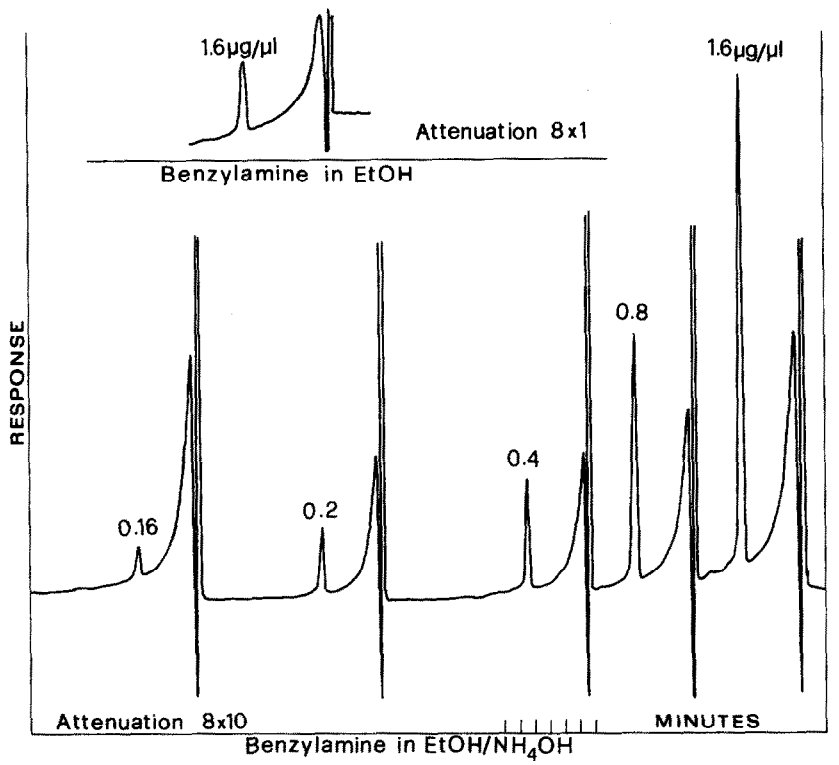


Figure 1
Gas chromatogram of standard solutions of benzylamine in ethanol with and without ammonia added. Injected volume 1 µl.

Table 1
Results obtained in a series of control analyses of lg samples of pure bethanidine with added benzylamine

Benzylamine		
Added (ppm)	Found (ppm)	Recovery (%)
500	480	96
250	240	96
100	100	100

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References

[1] S. G. Wood, M. R. Al-Ani and A. Lawson, *J. Pharm. Sci.* **68**, 374–376 (1979).
[2] H. I. Wase, Y. Takeuchi and A. Murai, *Chem. Pharm. Bull.* **27**, 1009–1014 (1979).
[3] P. S. Doshi and D. J. Edwards, *J. Chromatogr.* **176**, 359–366 (1979).
[4] C. R. Clark, J. D. Teague, M. M. Wells and J. H. Ellis, *Anal. Chem.* **49**, 912–915 (1977).
[5] M. Dalene, L. Mathiasson and J. Å. Jönsson, *J. Chromatogr.* **207**, 37–46 (1981).

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