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GAS CHROMATOGRAPHIC ANALYSIS OF MEDAZEPAM AND ITS METABOLITES USING AN ELECTROLYTIC CONDUCTIVITY DETECTOR

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SUMMARY

A Coulson detector has been used in the gas chromatography of medazepam and its major blood metabolites. In contrast to electron capture detection, an approximately equal response was obtained for each compound, and it was possible to temperature programme the column. Extracts from spiked samples of rat blood plasma gave clean chromatograms without the need for back extraction and clean up.

INTRODUCTION

There is at present widespread interest in the use of gas chromatography for the analysis of the 1,4-benzodiazepine drugs and their metabolites in body fluids. In the case of the widely used drug diazepam and the chemically similar compound medazepam, the most commonly used procedure involves the use of electron capture detection, following separation of the intact benzodiazepines on silicone stationary phases[1–5]. Quantification of these compounds at the low levels found following single therapeutic doses requires a clean-up procedure, using back extraction from dilute acid[2,5]. It is, however, possible to omit this lengthy stage when dealing with the relatively high levels found after chronic administration or overdose[3,4,6].

The electron capture detector has a relatively poor response to medazepam and its demethylated metabolite Ro 5-2925[2,5]. In addition, medazepam has a rather short retention time relative to the solvent front, and the peak resulting from this compound is susceptible to interference from solvent impurities and volatile co-extracted material. In the analysis of this compound it would obviously be advantageous to use a detection system that would have a greater response and that would be amenable to temperature programming. Mass spectrometry is an obvious possibility, but is prohibitively expensive for most establishments. Amongst the less expensive detectors, gas chromatography of the benzodiazepines using a thermionic detector was reported by Swann[7]. However, the detector design used in this work proved

unsuitable for use with biological samples and low levels of benzodiazepines[8]. Successful thermionic detection of the benzodiazepines in biological samples has been reported by Mallach *et al.*[9], who separated medazepam and its metabolites on an OV-25 column using a temperature programme. This method was applied to serum and urine samples although relatively large sample volumes were required.

Our approach to this problem has been to make use of an electrolytic conductivity detector based on the principle described by Coulson[10]. Use of dilute acid solutions has increased the sensitivity of this detector towards the nitrogen containing benzodiazepines[11].

MATERIAL AND METHODS

Apparatus

The apparatus used in this work consisted of a Pye 104 Series gas chromatograph to which an electrolytic conductivity detector had been fitted. The detector used in this work was essentially the same as the one used by Jones and Nickless in their work on the detection of pesticides and other complex organic chlorine compounds[11].

The reduction of the column eluates was performed by mixing the eluates with hydrogen (70 ml/min) and the gases were then passed over a nickel wire catalyst, which was heated to 850° in a microcombustion furnace.

Reagents

All reagents were obtained from BDH (Poole, Great Britain) and were of analytical grade. The benzodiazepines were obtained from Roche Products (Welwyn Garden, Great Britain).

Columns

The separations described in this work were achieved on two columns: (a) A 1 ft. × 4 mm. I.D. silanized glass column, packed with 3% OV-225 on Gas-Chrom Q (60–80 mesh). (b) A 6 ft. × 4 mm. I.D. silanized glass column, packed with 3% OV-17 on Gas-Chrom Q (60–80 mesh).

Experimental

It has been shown[12] that the major blood metabolites of medazepam are diazepam and the demethylated compounds Ro 5-2925 and Ro 5-2180 (Fig. 1). Each of these compounds contains one chlorine and two nitrogen atoms per molecule, and one mole of each of these is therefore expected to yield, on complete reduction, one mole of hydrogen chloride and two moles of ammonia.

Preliminary separation of medazepam and its metabolites was carried out on an OV-225 column. The detector response which was obtained using a 3-ppm hydrochloric acid solution[11] was consistent with a conductivity change due to a nitrogen-containing compound. When a boric acid scrubber was introduced between the furnace and the detector to remove ammonia from the gas stream, no response was obtained to the benzodiazepines, indicating that hydrogen chloride was not able to reach the conductivity cell. Good detector responses were still obtained to organochlorine pesticides with the boric acid scrubber in position. These preliminary results suggested that ammonium chloride was being formed downstream from the furnace,

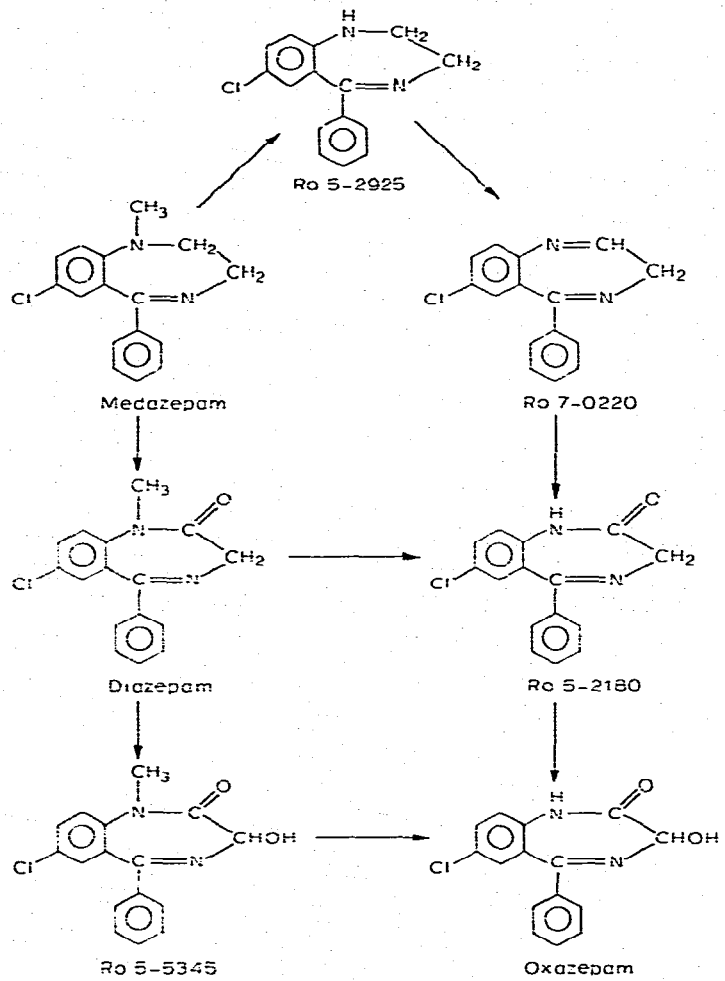

Fig. 1. The major metabolic pathways of medazepam.

and was condensing onto the tube walls as the temperature fell below the sublimation point of 335°. The residual ammonia was responsible for the observed conductivity change.

Standard solutions of medazepam and its metabolites desmethylmedazepam (Ro 5-2925), diazepam and desmethyldiazepam (Ro 5-2180) were prepared in acetone–hexane (20:80) and chromatographed on the OV-225 column, using a base liquid of 3 ppm hydrochloric acid in the conductivity cell. Linear detector response was obtained for all compounds over the range 10 to 500 ng. The detection limit for each compound was approximately 30 ng, this being the level which gave a response twice as large as the noise level.

A temperature-programmed separation of the four benzodiazepines was attempted on the OV-225 column, consisting of isothermal operation at 210° for 2 min followed by a temperature increase of 6°/min to 240°. The detector response was found to be dependent on the column temperature. The resistance of the base liquid

was found to decrease with column temperature when water was used, and increase in the case of dilute (3 ppm) hydrochloric acid. This indicated an increase in the rate of ammonia dissolution in the liquid, due to bleed from the stationary phase. This was not unexpected as OV-225 is a cyanopropylsilicone polymer. Removal of the bleed using a scrubber was clearly not feasible as this would have prevented detection of the benzodiazepines. The scrubber would in any case have been rapidly saturated.

To overcome the problem of nitrogenous bleed, chromatography was carried out on the methylphenylsilicone OV-17, using a carrier gas (argon) flow-rate of 50 ml/min, and an identical temperature programme. A satisfactory baseline was obtained, and the programme was capable of separating medazepam from the solvent front.

Application to biological samples. The major advantage in the use of the electrolytic conductivity detector is the selectivity which it offers. If dilute acid is used as the base liquid, then unique response to nitrogen-containing compounds can be obtained[11]. The Coulson detector should render lengthy extraction and clean-up procedures unnecessary. The application of this device to the analysis of drugs in body fluids was evaluated by the chromatographic analysis of extracts from spiked rat plasma samples.

Method

A 25-ml sample of rat blood plasma was spiked with medazepam, diazepam, Ro 5-2925 and Ro 5-2180 to give a solution of concentration 2 μg/ml for each compound. The plasma was buffered to pH 9.0 with 10 ml of borate buffer and extracted with two 10-ml aliquots of diethyl ether. The combined extracts were evaporated to dryness and the residue was dissolved in 100 μl of acetone–hexane (1:4). One μl of this solution was chromatographed.

RESULTS

Chromatograms that were obtained under isothermal and temperature-programmed conditions are shown in Fig. 2. It can be seen that, under isothermal conditions, the medazepam peak is close to the solvent peak. Although no major

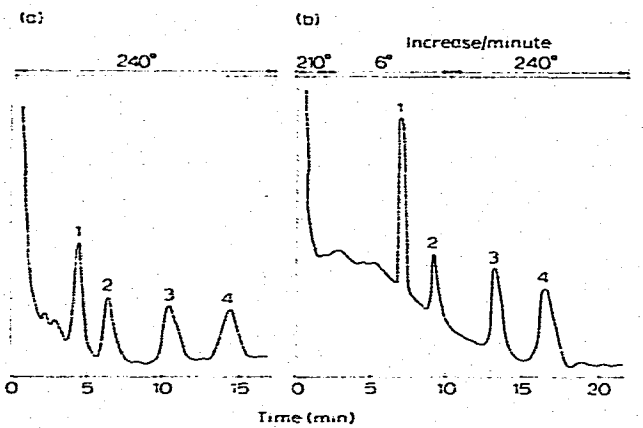

Fig. 2. Isothermal (a) and temperature-programmed (b) separations of medazepam and its major metabolites. 1 = Medazepam; 2 = Ro 5-2925; 3 = diazepam; 4 = Ro 5-2180.

problem is presented in this case, it is desirable that the medazepam peak should be moved away from the solvent peak by temperature programming. When this was carried out the medazepam peak was moved away from the solvent peak but a sloping baseline was obtained. The reason for this is uncertain as standard solutions gave chromatograms with level baselines. The magnitude of this effect is not, however, considered large enough to preclude the use of this technique for clinical samples.

DISCUSSION

The sensitivity of the present device to medazepam and its metabolites may be improved by the use of a quartz gas–liquid contactor which could be heated above the sublimation temperature of ammonium chloride. In addition, pilot experiments have shown that greater sensitivity to these benzodiazepines can be obtained by performing the hydrogenation over a platinum rather than a nickel catalyst. This results in the production of hydrogen chloride only, which gives rise to a larger conductivity change than an equal quantity of ammonia[13]. The most promising mode of operation of the Coulson detector for the detection of medazepam and its metabolites will probably be found by using the detector in the oxidative mode, using high purity water as base liquid. The response of the detector in the oxidative mode should be due to both the chlorine and nitrogen atoms in the benzodiazepines, and greater sensitivity should therefore be obtained.

The nickel catalyst reduction system should prove more suitable for the 7-nitrobenzodiazepine, nitrazepam, which contains no halogen atom in the molecule. The main metabolites of this compound result from the reduction of the nitro group, and are not strongly electron capturing[14]. Use of the conductivity detector for the analysis of nitrazepam and its metabolites should therefore have advantages over the electron capture detector in terms of response to the 7-amino and 7-acetamido metabolites. Response of the conductivity detector to the nitrazepam system has been studied by Hunt[15], who found that the detector gave similar responses to nitrazepam and its 7-amino metabolite.

CONCLUSIONS

The detection of medazepam and its metabolites by the electrolytic conductivity detector has been investigated and the anomalous response to these compounds has been explained. It has been demonstrated that, unlike the electron capture detector, the electrolytic conductivity detector can be usefully employed for temperature programmed separations, provided that the chromatographic column is correctly chosen.

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REFERENCES

1 F. Marcucci, R. Fanelli and E. J. Mussini, *J. Chromatogr.*, 37 (1968) 318.
2 J. A. F. de Silva and C. V. Puglisi, *Anal. Chem.*, 42 (1970) 1725.

3 I. A. Zingales, *J. Chromatogr.*, 75 (1973) 55.
4 A. Berlin, B. Sivers, S. Agurell, A. Hiort, F. Sjoqvist and S. Strom, *Clin. Pharmacol. Ther.*, 13 (1972) 733.
5 E. S. Baird, D. M. Hailey and S. L. Malcolm, *Clin. Chim. Acta*, 48 (1973) 105.
6 A. G. Howard, G. Nickless and D. M. Hailey, *J. Chromatogr.*, 90 (1974) 325.
7 D. Swann, *Column*, 14 (1972) 9.
8 J. H. Goudie, personal communication.
9 H. J. Mallach, A. Moosmayer and J. M. Rupp, *Arzneim.-Forsch.*, 23 (1973) 614.
10 D. M. Coulson, *J. Gas Chromatogr.*, 4 (1966) 285.
11 P. Jones and G. Nickless, *J. Chromatogr*, 73 (1972) 19.
12 M. A. Schwartz and J. J. Carbone, *Biochem. Pharmacol.*, 19 (1970) 343.
13 W. P. Cochrane, B. P. Wilson and R. Greenhalgh, *J. Chromatogr.*, 75 (1973) 207.
14 G. P. Beharrell, D. M. Hailey and M. K. McLaurin, *J. Chromatogr.*, 70 (1972) 45.
15 L. M. Hunt, *M. Sc. Thesis*, University of Bristol, Bristol 1972.