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Note

Decomposition of sulphoxide metabolites of phenothiazine antipsychotics during gas chromatographic analysis

K. HALL, P.K.F. YEUNG and K.K. MIDHA*

College of Pharmacy, University of Saskatchewan, Saskatoon, Saskatchewan S7N OWO (Canada)

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During a recent evaluation of a gas chromatographic and gas chromatographic—mass spectrometric (GC—MS) assay for the psychotherapeutic agent chlorpromazine and its major metabolites, it was noted that the sulphoxide metabolite decomposed upon injection into the gas chromatograph to give a small but significant peak with the same retention time as that of the parent drug chlorpromazine. Decomposition of the N-oxide metabolite during GC analysis has been reported [1]. However, information as to the stability of the sulphoxide metabolite in GC analysis is lacking in spite of the fact that there are reports which describe GC procedures for chlorpromazine and its metabolites including the sulphoxide metabolite [2, 3]. Thus it was decided to examine the effect of injection port temperature on the extent of decomposition of phenothiazine sulphoxide metabolites during GC analysis, using both capillary and packed columns with mass spectrometric and nitrogen sensitive detection.

EXPERIMENTAL

All solvents were redistilled in glass before use. The samples of chlorpromazine sulphoxide (CPZSO) (synthesized in our laboratory), trifluorperazine sulphoxide (TFPSO) and prochlorperazine sulphoxide (PCPSO) (provided by courtesy of Rhone Poulenc, Montreal, Canada) were purified by thin-layer chromatography (TLC) (Whatman TLC plates precoated with 40A silica gel, 250 μm) with a solvent system acetone—methanol—diethylamine (100:20:1). The area on the TLC plate corresponding to the sulphoxides (R_F 0.16 for PCPSO, R_F 0.22 for TFPSO and R_F 0.38 for CPZSO) were scraped off and the sulphoxides were redissolved in methanol. The purity of the samples was then confirmed by high-performance liquid chromatography using a Waters M45 pump system and Waters Model 440 UV detector operating at 254 nm wavelength. A 250 mm × 4.6 mm I.D. column packed with 5 μm

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Spherisorb Cyano was used with acetonitrile—0.01 *M* acetate buffer (9:1) as solvent system. Injection was made via a Rheodyne loop Model 7125 fitted with a 500 μl loop.

Gas chromatography with nitrogen selective detection was performed on a Hewlett-Packard 5840 series gas chromatograph fitted with a 1.5 m × 2 mm I.D. glass column packed with Gas-Chrom Q (100—120 mesh) (Applied Science). The injection port was operated at either 250, 270 or 300°C, the column oven was maintained isothermally at 290°C. The helium flow-rate was set at 50 ml/min.

Capillary column GS—MS was performed using a V.G. Analytical 16F single focussing mass spectrometer coupled to a Hewlett-Packard 5700 gas chromatograph via a direct glass capillary coupling to the ion source. A 20 × 0.3 mm I.D. fused silica capillary column coated with methyl silicone OV-1 was used. Injections were made in the split and splitless modes with an injector temperature at 300°C, and with the cold on column injection mode [4]. The column was programmed from 60 to 280°C at 6°C/min in each case. Additional mass spectrometer operating conditions were interface temperature 280°C, ion source 200°C, ion energy 70 eV, emission current 200 μA in the electron impact mode and 500 μA in the chemical ionisation mode using ammonia as reagent gas.

RESULTS AND DISCUSSION

Gas chromatographic analysis of chlorpromazine sulfoxide using a packed column at an injection port temperature of 300°C showed two peaks a and b (Fig. 1A) with a retention time of 1.5 and 4.5 min, respectively. When the injection port temperature was lowered, peak b increased at the expense of peak a. Peak a had the same retention time as authentic chlorpromazine. Both the

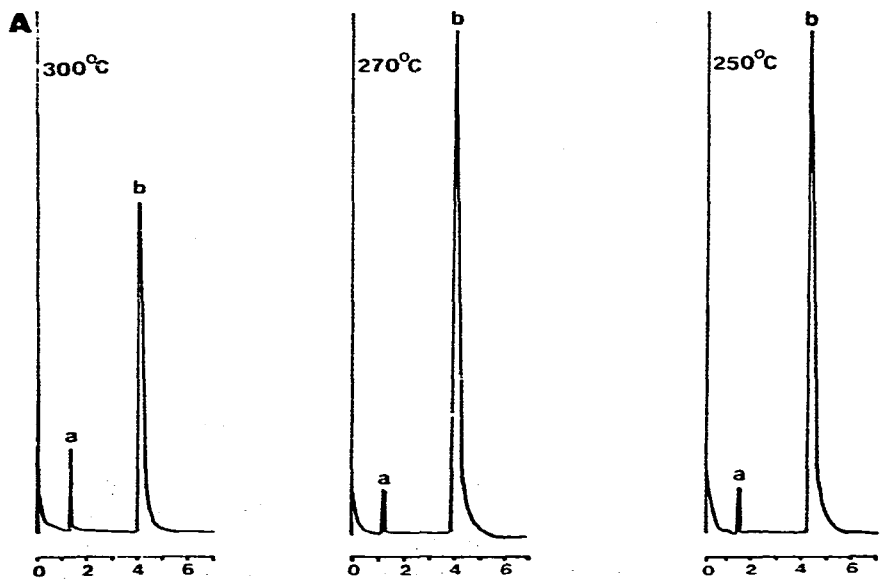

Fig. 1.

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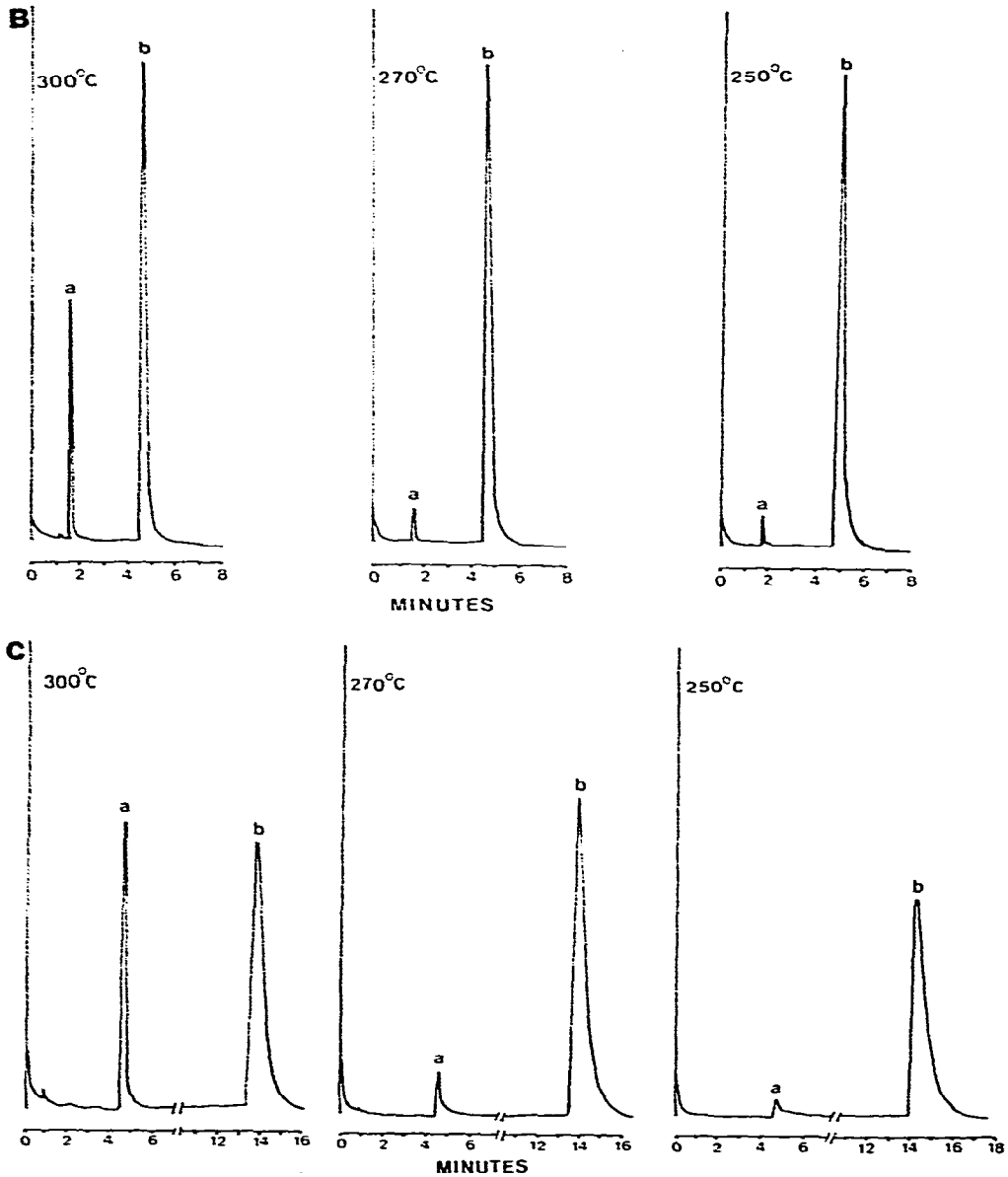

Fig. 1. Typical gas—liquid chromatograms of CPZSO (A), TFPSO (B) and PCPSO (C) at three different injection port temperatures. Peak a is the parent drug and peak b is the respective sulphoxide, in each case.

electron impact (EI) and chemical ionisation (CI) mass spectra of the compound giving rise to peak a were identical to those of authentic chlorpromazine. The EI mass spectrum showed the molecular ion at *m/z* 318/320, the base peak at *m/z* 58 and other diagnostic ions at *m/z* 273/275; 272/274; 233/235; 196, 86 and 85. The mass spectra of the compound giving rise to peak b were consistent with the structure of authentic chlorpromazine sulfoxide. The EI mass spectrum showed a molecular ion cluster at *m/z* 334/336, and other

diagnostic ions at *m/z* 318/320 and *m/z* 317/319. The CI mass spectrum using ammonia reagent gas showed the quasi molecular ion at *m/z* 335/337 $(M+H)^+$. Similar to the GC behavior of CPZSO, decomposition of TFPSO and PCPSO to their respective phenothiazines also occurred in GC (Fig. 1B and C), and was also confirmed by EI and CI MS. The extent of decomposition for these two sulfoxide compounds, however, was more pronounced than in the case of CPZSO.

Results from the injection of the phenothiazine sulfoxides made at three different injection port temperatures using nitrogen selective GC detection, and also capillary column GC—MS using split, splitless and cold on column injection [4] are presented in Table I.

TABLE I

PERCENT DECOMPOSITION OF CPZSO, PCPSO, AND TFPSO IN THE GAS CHROMATOGRAPH AT DIFFERENT INJECTION PORT TEMPERATURES

Each value represents the mean of 3 injections.

Column	Injection port temperature (°C)	Decomposition (%)		
		CPZSO	PCPSO	TFPSO
Packed (1.5 m × 2 mm I.D.) 280°C	300	10	24	20
	270	2	8	2
	250	2	2	2
Capillary (20 m × 0.3 mm I.D.) 60—280°C at 6°C/min	Split 300	8	15	17
	Splitless 300	8	16	18
	Cold on column	0.3	0.5	0.7

It can be easily observed from Fig. 1 and Table I that injection port temperature has a marked effect on the extent of decomposition. In the case of cold on column method of injection, the break down was minimal, although this method of injecting samples is not practical in routine analysis.

Although the decomposition of chloropromazine N-oxide in GC was noted as early as 1964 [1], we are unaware of any reports regarding the decomposition of the sulfoxide metabolite, despite the fact that the injection port temperatures in most reported GC analysis of CPZ and its metabolites were in the range of 275—310°C. The sulfoxide metabolites, although they are thought to be relatively inactive therapeutically [5], may be present in plasma at levels similar to or even exceeding that of the parent drugs [6, 7]. Thus a significant contribution may be made to the concentrations of the parent compounds by thermal breakdown.

To minimize this analytical complication, we would suggest careful optimisation of chromatographic conditions prior to the analysis of phenothiazine sulfoxides. It was found that by maintaining the injection port temperature

between 250—270°C, the decomposition rarely exceeded 2% for the three sulfoxide metabolites tested, and the chromatographic behavior of these metabolites was not changed by the lowering of the injection port temperature within this range.

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REFERENCES

1 J.C. Craig, N.Y. Mary and S.K. Roy, Anal. Chem., 36 (1964) 1142.
2 S.H. Curry, Psychopharmacol. Commun., 2 (1976) 1.
3 G. Alfredsson, B. Wode-Helgodt and G. Sedvall, Psychopharmacol., 48 (1976) 123.
4 K. Grob, Jr. and H. Neukom, J. High Resol. Chromatogr. Chromatogr. Commun., 2 (1979) 15.
5 L.J. Traficante, G. Sakalis, J. Siekierski, J. Rotrosen and S. Gershon, Life Sci., 24 (1979) 337.
6 O.T. Phillipson, J.M. McKeown, J. Baker and A.F. Healy, Brit. J. Psychiat., 131 (1977) 172.
7 A.V.P. MacKay, A.F. Healy and J. Baker, Brit. J. Clin. Pharmacol., 1 (1974) 425.