Journal of Chromatography, 538 (1991) 277–284
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 851

Collection of analytical data for benzodiazepines and benzophenones

Appendix

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(First received June 13th, 1990; revised manuscript received September 25th, 1990)

ABSTRACT

An analytical database is presented for six benzodiazepines (clotiazepam, delorazepam, ethyl loflazepate, fludiazepam, haloxazolam and oxazolam) which are controlled in the U.K. Chromatographic, ultraviolet spectroscopic and mass spectrometric data are presented. Analytical data are also included for the benzophenones which have been prepared from the benzodiazepines.

INTRODUCTION

An earlier publication [1] detailed analytical data for twenty-seven of the thirty-three benzodiazepines which are controlled in the U.K. At the time of preparing the paper [1], six of the controlled benzodiazepines (clotiazepam, delorazepam, ethyl loflazepate, fludiazepam, haloxazolam and oxazolam) were unavailable for inclusion in the paper. Reference samples of these six drugs have now been obtained and an analytical database is presented as an appendix to the previous paper. Chromatographic properties have been measured including gas chromatography (GC) retention index (I) values, high-performance liquid chromatography (HPLC) capacity factors (k') and thin-layer chromatography (TLC) $R_F \times 100$ values. Ultraviolet (UV) spectroscopic data and mass spectrometric (MS) data are also given. Benzophenones have been prepared from the parent benzodiazepines and their GC, TLC and MS properties are also described.

EXPERIMENTAL

Materials

The benzodiazepines were from the drug collection of the Central Research and Support Establishment, Home Office Forensic Science Service. Benzophenones were prepared from the parent benzodiazepines by acid hydrolysis for 1 h followed by extraction into diethyl ether [2].

Methods

GC, HPLC, TLC and UV. The experimental conditions employed were identical to those described previously [1]. For HPLC and TLC, mixtures containing benzodiazepines and benzophenones, for which data had been obtained for the previous report, were run simultaneously with the six new benzodiazepines and their benzophenones. The measured capacity factors (k') and $R_F \times 100$ values of these new compounds were then corrected to standardise all data in the collection. For TLC of benzodiazepines, the $R_F \times 100$ values were corrected using the reference compounds described by Stead *et al.* [3].

For UV spectrophotometry, $E^{1\%}_{1cm}$ values were only calculated for those benzodiazepines which were completely soluble.

MS. The mass spectrometer was a VG Masslab Quadrupole 12-250 instrument interfaced to a PDP 11/73 data system.

Probe spectra of the benzodiazepines were collected over a mass range from 20 to 500 a.m.u. with a scan speed of 3 s per decade and a 0.1-s inter-scan delay.

GC–MS analysis of benzodiazepines and benzophenones was performed using a Hewlett-Packard 5890 gas chromatograph fitted with a DB1 narrow-bore capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). Spectra were collected over a mass range from 20 to 500 a.m.u. with a scan speed of 1 s per decade and a 0.1-s inter-scan delay.

All other conditions were identical to those described previously [1].

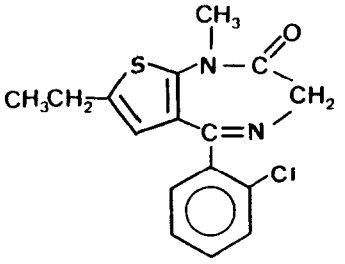
RESULTS AND DISCUSSION

The chemical structures of the benzodiazepines are shown in Fig. 1. The benzophenones produced by acid hydrolysis of the parent benzodiazepines are listed alphabetically by chemical name in Table I and their chemical structures are shown in Fig. 2. Clotiazepam did not produce a benzophenone after acid hydrolysis; two products were obtained in minor quantities, detected by GC–MS (see Table VIII).

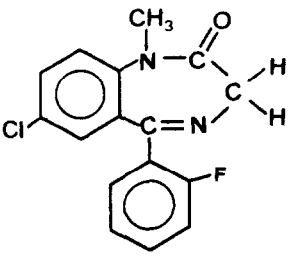
Table II lists the GC I values of the benzodiazepines and benzophenones on SE-30 equivalent narrow-bore and wide-bore capillary columns. Three of the benzodiazepines (ethyl loflazepate, haloxazolam and oxazolam) gave multiple peaks by decompositon and/or rearrangement. These benzodiazepines were not easily analysed by GC and concentrated solutions of the drugs were required for detection using a flame ionisation detector. The chromatograms obtained for ethyl loflazepate and haloxazolam were particularly complex. For ethyl loflazepate, the I values of the two major peaks only are given. Severe peak tailing was obtained for haloxazolam and the late eluting peak (I 2684) was very broad.

A list of HPLC k' values for benzodiazepines measured using four HPLC systems (A, B, C and D) is presented in Table III. Systems A and B (ODS-silica) contained 55% and 70% (v/v) methanol, respectively, to cover the wide range of polarities of benzodiazepines. System B was required to elute the more hydrophobic benzodiazepines, therefore only those benzodiazepines the k' values of which with system A were greater than 9 were eluted using system B. Haloxazolam and oxazolam each produced two peaks on the ODS-silica column; with the silica column only one peak was observed from each of these compounds. The multiple peaks obtained for haloxazolam and oxazolam are most likely due to decomposition, as pure samples of these drugs were received from the manufacturers.

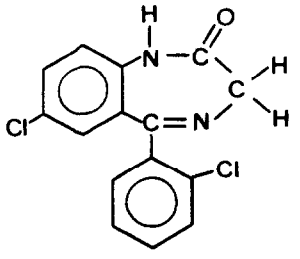
Clotiazepam



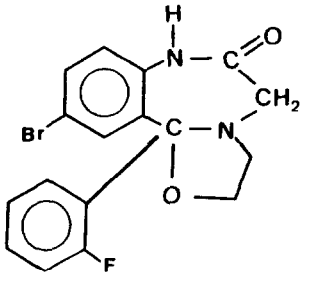
Fludiazepam



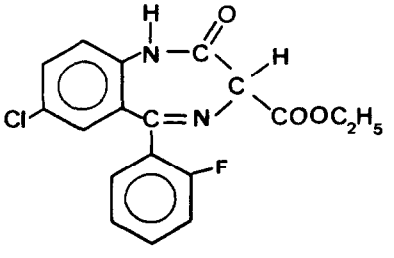
Delorazepam



Haloxazolam



Ethyl loflazepate



Oxazolam

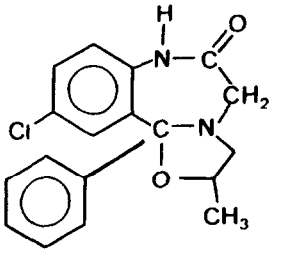
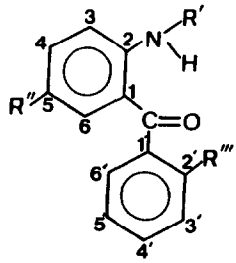


Fig. 1. Structures of benzodiazepines.

The TLC $R_F \times 100$ values for the benzodiazepines measured on three TLC systems are given in Table IV. The experimentally determined $R_F \times 100$ values have been corrected against the standard $R_F \times 100$ values of reference compounds [3] and it is the corrected values which are presented in Table IV. Haloxazolam and oxazolam gave multiple spots on the TLC plates and with system 3, tailing bands were obtained



	R'	R"	R'''
ABFB :	H	Br	F
ACB :	H	Cl	H
ACFB :	H	Cl	F
ADCB :	H	Cl	Cl
MCFB :	CH_3	Cl	F

Fig. 2. Structures of benzophenones. For abbreviations see Table I.

for these compounds (probably due to decompositon). The benzodiazepines gave a yellow-brown or orange-brown colour with acidified iodoplatinate solution, except for ethyl loflazepate, which gave no reaction with this locating reagent.

For the benzophenones, the TLC $R_F \times 100$ values were measured on four TLC systems and the experimentally determined values are given in Table V. The reaction of the benzophenones to the Bratton–Marshall test [5] is also included.

Table VI contains ultraviolet absorption data for the benzodiazepines measured in acid, alkali and ethanol. The λ_{max} values and points of inflexion are presented and

TABLE I

BENZOPHENONES PRODUCED BY HYDROLYSIS OF BENZODIAZEPINES

Benzophenone	Abbreviation	Parent benzodiazepine
2-Amino-5-bromo-2'-fluorobenzophenone	ABFB	Haloxazolam
2-Amino-5-chlorobenzophenone	ACB	Oxazolam
2-Amino-5-chloro-2'-fluorobenzophenone	ACFB	Ethyl loflazepate
2-Amino-5,2'-dichlorobenzophenone	ADCB	Delorazepam
2-Methylamino-5-chloro-2'-fluorobenzophenone	MCFB	Fludiazepam
No benzophenone produced		Clotiazepam

TABLE II

GC RETENTION INDICES FOR BENZODIAZEPINES AND BENZOPHENONES ON SE-30 EQUIVALENT NARROW-BORE AND WIDE-BORE CAPILLARY COLUMNS

For compounds giving multiple peaks, minor peaks are shown in brackets.

Benzodiazepine or benzophenone	*I*	
	Narrow-bore capillary column	Wide-bore capillary column
2-Amino-5-bromo-2'-fluorobenzophenone (ABFB)	2079	2090
2-Amino-5-chlorobenzophenone (ACB)	2005	2028
2-Amino-5-chloro-2'-fluorobenzophenone (ACFB)	1987	2004
2-Amino-5,2'-dichlorobenzophenone (ADCB)	2128	2141
Clotiazepam	2513	2530
Delorazepam	2571	2593
Ethyl loflazepate	2442[a] (3010)	2439, 2935
Fludiazepam	2389	2408
Haloxazolam	2560[a] (2302[b], 2684[b])	2572[a] (2323[b], 2695[b])
2-Methylamino-5-chloro-2'-fluorobenzophenone (MCFB)	2060	2055
Oxazolam	2564 (2586)	2569 (2596)

[a] Peak tailing.
[b] Severe peak tailing.

for those benzodiazepines completely soluble in aqueous solution or ethanol, the $E^{1\%}_{1cm}$ values have been determined for the wavelengths of maximum absorbance.

The GC–electron ionisation (EI)-MS data for benzodiazepines and benzophenones are given in Tables VII and VIII, respectively, as listings of the eight most intense ions and relative intensities. Also included in Table VII is a similar listing for

TABLE III

HPLC CAPACITY FACTORS FOR BENZODIAZEPINES ON FOUR HPLC SYSTEMS

Systems: A = methanol–water–phosphate buffer (0.1 *M*) (55:25:20, v/v/v), ODS-Hypersil; B = methanol–water–phosphate buffer (0.1 *M*) (70:10:20, v/v/v), ODS-Hypersil; C = methanol (1000 ml) containing perchloric acid (100 μl), Spherisorb S5W; D = methanol–water–trifluoroacetic acid (997:2:1, v/v/v), Spherisorb S5W.

Benzodiazepine	*k'*			
	System A	System B	System C	System D
Clotiazepam	15.8	2.87	1.89	4.15
Delorazepam	6.46	–	0.85	1.04
Ethyl loflazepate	12.50	2.17	0.08	0.08
Fludiazepam	6.70	–	0.99	1.25
Haloxazolam	6.80, 10.75	2.13	2.19	5.40
Oxazolam	19.25, 22.95	3.48, 3.87	1.77	4.05

TABLE IV

TLC R_F × 100 VALUES FOR BENZODIAZEPINES ON THREE TLC SYSTEMS

Systems: 1 = cyclohexane–toluene–diethylamine (75:15:10, v/v/v), TLC plates pre-treated with methanolic KOH (0.1 *M*) [1]; 2 = chloroform–methanol (90:10, v/v), TLC plates pre-treated with methanolic KOH (0.1 *M*) [1]; 3 = chloroform–acetone (80:20, v/v).

Benzodiazepine	R_F × 100 value		
	System 1	System 2	System 3
Clotiazepam	33	70	53
Delorazepam	45	54	35
Ethyl loflazepate	0	58	50
Fludiazepam	23	70	51
Haloxazolam	3, 10	47, 66	44, 23 (12-32)[a]
Oxazolam	3, 11, 15	56,68	51[b]

[a] Broad tailing band.
[b] Tailing.

TABLE V

TLC R_F × 100 VALUES FOR BENZOPHENONES ON FOUR TLC SYSTEMS

Systems: 4 = toluene; 5 = toluene–isopropanol–ammonia (sp. gr. 0.880) (85:15:1, v/v/v); 6 = chloroform–methanol (90:10, v/v); 7 = chloroform–acetone (80:20, v/v). NR = No reaction. For abbreviations see Table I.

Benzophenone	R_F × 100 value				Reaction to Bratton–Marshall test
	System 4	System 5	System 6	System 7	
ABFB	20	63	70	63	Purple
ACB	16	63	70	64	Purple
ACFB	19	63	69	62	Purple
ADCB	21	63	70	63	Purple
MCFB	35	70	76	66	NR

TABLE VI

ULTRAVIOLET ABSORPTION DATA FOR BENZODIAZEPINES

Specific absorbance values are shown in parentheses after the corresponding λ_{max} value. ND = Not determined, insoluble.

Benzodiazepine	λ_{max} (nm) and specific absorbance measured in 0.1 *M* sulphuric acid	λ_{max} (nm) and specific absorbance measured in 0.1 *M* sodium hydroxide	λ_{max} (nm) and specific absorbance measured in absolute ethaol
Clotiazepam	214 (686), 261 (618), 302[a], 392 (153)	239, 351	212 (914), 243 (690), 319 (89)
Delorazepam	239, 286, 366	230, 274[a], 343	228 (1165), 257[a], 320
Ethyl loflazepate	ND	235 (852), 346 (70)	229 (1072), 318
Fludiazepam	240 (929), 283 (389), 364 (109)	233, 250[a], 312	229 (1086), 253[a], 316
Haloxazolam	243 (837), 286, 375	ND	247 (402)
Oxazolam	238 (930), 281 (272), 306[a], 369 (98)	ND	245 (422)

[a] Point of inflexion.

TABLE VII

MOLECULAR WEIGHTS AND EIGHT MOST INTENSE IONS OBSERVED USING EI CONDITIONS FOR BENZODIAZEPINES

Benzodiazepine	Molecular weight	*m/z* (% intensity)	
		Probe (EI)	Capillary GC (EI)
Clotiazepam	318	289 (100), 318 (56), 291 (42), 275 (32) 290 (23), 39 (22), 320 (20), 45 (16)	289 (100), 318 (64), 291 (38), 320 (24) 290 (24), 275 (23), 319 (16), 283 (15)
Delorazepam	305	275 (100), 269 (97), 277 (90), 304 (84) 303 (67), 276 (65), 306 (63), 305 (56)	275 (100), 269 (93), 304 (86), 277 (83) 36 (75), 303 (62), 276 (60), 306 (58)
Ethyl loflazepate	360	259 (100), 287 (54), 288 (46), 261 (38) 260 (28), 289 (23), 223 (17), 286 (14)	260[a] (100), 259 (100), 288 (74), 287 (67) 216 (67), 75 (62), 102 (49), 262 (38)
Fludiazepam	302	274 (100), 302 (81), 301 (81), 275 (46) 273 (41), 303 (40), 276 (35), 283 (33)	274 (100), 301 (85). 302 (79), 275 (48) 273 (44), 303 (40), 276 (38), 283 (33)
Haloxazolam	377	283 (100), 281 (100), 206 (33), 123 (24) 56 (17), 282 (16), 284 (15), 95 (12)	43[a] (100), 295 (50), 293 (50), 294 (42) 292 (36), 42 (26), 213 (13), 276 (12)
			305[a] (100), 303 (99), 304 (39), 209 (35) 182 (32), 183 (29), 306 (22), 181 (22)
			305[a] (100), 226 (78), 307 (49), 303 (49) 276 (36), 278 (34), 197 (31), 183 (27)
			210[a] (100), 333 (83), 335 (81), 183 (74) 211 (73), 291 (52), 290 (49), 289 (47)
Oxazolam	328	251 (100), 253 (42), 70 (33), 252 (19) 77 (18), 105 (17), 283 (11), 42 (11)	241[a] (100), 243 (36), 191 (29), 242 (23) 240 (23), 102 (23), 164 (20), 103 (19)
			251[a] (100), 70 (45), 253 (36), 42 (22) 77 (21), 41 (19), 105 (18), 252 (15)

[a] Decomposition product.

TABLE VIII

MOLECULAR WEIGHTS AND EIGHT MOST INTENSE IONS OBSERVED USNG EI CONDITIONS FOR BENZOPHENONES

For abbreviations of benzophenones, see Table I.

Benzophenone	Molecular weight	*m/z* (% intensity), capillary GC (EI)
ABFB	294	293 (100), 295 (97), 123 (88), 294 (79) 292 (67), 95 (63), 91 (34), 63 (34)
ACB	231	230 (100), 231 (74), 77 (54), 232 (43) 105 (30), 233 (25), 154 (22), 126 (17)
ACFB	249	249 (100), 248 (86), 123 (57), 95 (44) 154 (42), 250 (41), 251 (34), 126 (28)
ADCB	265	230 (100), 265 (52), 139 (40), 267 (34) 154 (34), 232 (33), 111 (33), 126 (25)
MCFB	263	263 (100), 246 (66), 211 (61), 262 (50) 265 (41), 95 (37), 123 (36), 264 (35)
Clotiazepam acid hydrolysis products		
1		111 (100), 264 (92), 139 (75), 279 (71) 244 (65), 229 (62), 228 (58), 152 (54)
2		255 (100), 284 (58), 256 (30), 283 (20) 241 (19), 198 (13), 77 (13), 285 (11)

benzodiazepines, from spectra obtained after sample introduction via the direct insertion probe.

Thermal degradation occurred during the capillary GC–MS analysis of some of the benzodiazepines [4]. The spectra of the decomposition products are also listed in Table VII. For ethyl loflazepate, haloxazolam and oxazolam, concentrated solutions of the drugs were required to obtain mass spectra. The GC–MS analysis of haloxazolam was especially complex and four spectra were obtained for the major components from this compound. For ethyl loflazepate, GC–MS analysis gave only one mass spectrum; the molecular ion of the parent compound (*m/z* 360) was not observed.

Also included in Table VIII are the relative intensities of the eight most intense ions for the two products from the acid hydrolysis of clotiazepam.

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