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ANALYTICAL STUDIES OF SOME CONTROLLED AND NONCONTROLLED DRUGS OF ABUSE (II)

By

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دراسة كيميائية تحليلية على بعض العقاقير الخاضعة وغير الخاضعة للرقابـة (٢)

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تشتمل هذه الدراسة على مقارنة سلوك بعض العقاقير التي يساء استخدامها سواء كانت خاضعة أو غير خاضعة للرقابة وعلى الأخص مركبات البنزوديازيبينات ، وذلك باستخدام طرق تحليل آلية مثل كروماتوجرافيا الغاز وكروماتوجرافيا السائل عالية الكفاءة .

ولقد أوضحت الدراسة أن كلاً الوسيلتين مكملتين في عمليات التعرف والتقدير للمواد المخدرة .

Key Words: Depressants, Benzodiazepines, Barbiturates, Non-barbiturate hypnotics, Stimulants, Xanthine derivatives, Amphetamines. GLC, HPLC, Comparison.

ABSTRACT

Benzodiazepines, barbiturates, amphetamines and some other non-controlled drugs were identified by GLC and HPLC then confirmed by GC-MS techniques. Comparative studies and discussions on the results obtained by GLC and HPLC were attempted.

INTRODUCTION

In previous work controlled and noncontrolled drugs of abuse were separated and identified by gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC). (Mobarak *et al.*, 1991).

A sensitive and specific GLC was developed for the determination of nitrazepam (Moeller Jensen, 1975), clonazepam, flunitrazepam (Cano *et al.*, 1977) and oxazepam (Yalabik-Kas, 1983) in biological fluids as their benzophenones. The detection limit ranged from 0.5-20 ng/ml. After butylation of diazepam metabolites they were detected at the nanogram level in blood plasma (De Geir and 't. Hart, 1979). Electroncapture gas chromatography was applied for identification and quantitative determination of 11 benzodiazepines and 12 metabolites (Hoene *et al.*, 1987) Reversed phase high performance liquid chromatography method for determination of chlordiazepoxide and its metabolites in plasma was achieved (Ascalone, 1980). Diazepam and its metabolites were determined in serum by HPLC (Ratnaraj *et al.*, 1981; Tada *et al.*, 1985). Nitrazepam and its metabolites are well determined by HPLC (Tada *et al.*, 1987). The behaviour of the different drugs using such techniques was studied by several authors (Japp *et al.*, 1986; Flanagan *et al.*, 1982; Nobuhare *et al.*, 1980; Smith 1976; Hermann 1976; Zaki *et al.*, 1980; Mobarak *et al.*, 1991; Somokin 1973; Szendrei 1980).

EXPERIMENTAL

Substances used: Authentic samples of controlled substances were obtained from Medicolegal Labs., Ministry of Justice, Cairo, Egypt, in concentration non exceed 1 mg/1 ml. Noncontrolled substances are found in our Labs.

All studied samples are examined and analysed by GC-MS techniques (as in ref. Mobarak *et al.*, 1991).

Gas Liquid Chromatography

Gas chromatograph, Fractovap series 4200 Carlo Erba, attached with FID and programmer Model 400 was used.

Integrator: Computarized C-R3A chromatopac Shimadzu.

Column: Capillary column 30 m (0.32 I.D.) filled with DB/5 of film thickness 0.25 mm.

Column temp.: programmed temp.: 80-265° C, (9° C/min.) with 2 minutes initial delay and 10 minutes isothermal (after reaching 265°C).

Injection temp.: 270° C.

Detector temp.: 270°C.

Carrier gas: Nitrogen 4 ml/min.

Gas Chromatography-Mass Spectrometry

Apparatus: Finnigen 4023

Column: Capillary column 30 m (0.32 I.D.) filled with DB/5 film thickness 0.25 um.

Column temp.: programmed temp.: 120-270° C, 2 minutes initial 8° C/min.

Carrier gas: Helium 0.8 ml/min.

Injection temp.: 250° C.

Electron energy: 70 eV.

High Performance Liquid Chromatography (HPLC)

Reversed phase method was used.

Apparatus: Gynkotec gradient former model B
Gynkotech constant flow pump model 600/200
Degasser REC-3520
Spectroflow monitor SF 770
GM monochromator wavelength drive SFA 339.

Column: 250 mm (5 mm I. D.)

Packing material: Hypersil 5 ODS

Mobile phase (Methanol): (Methanol/H2O 1:1) 50/50 v/v

Flow rate: 0.8 ml/min.

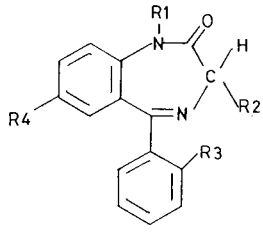
Detector: UV at 253 nm.

Inj. volum: 20 ul.

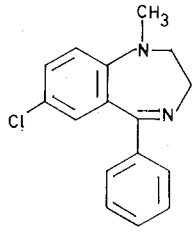
RESULTS AND DISCUSSION

Gas-Liquid Chromatography

Table 1
Gas chromatographic data of some benzodiazepines



Benzodiazepine



Medazepam

	R1	R2	43	R4	Relative retention time
Medazepam	-CH_3	-H	-H	-Cl	118.0
Diazepam	-CH_3	-H	-H	-Cl	131.9
Nordazepam	-H	-H	-H	-Cl	136.5
Oxazepam	-H	-OH	-H	-Cl	125.4
Lorazepam	-H	-OH	-Cl	-Cl	130.3
Nitrazepam	-H	-H	-H	$-NO_2$	154.1
Clonazepam	-H	-H	-Cl	$-NO_2$	160.7

From Tables (1) and (2), it is clear that the different types of drugs were separated by GLC and the sequance of separation is phenylalkylamines (amphetamine 26.2, methamphetamine 27.2, norephedrine 44.6 and L-ephedrine 49.6), barbituric acid derivatives (sandoptal 77.0, amytal 81.0 and seconal 86.5); xanthines CNS stimulants (caffeine 89.2, theophylline 103.7), cocaine 116.6 and benzodiazepines (118.7-160.7).

Regarding benzodiazepine separation, it is clear that medazepam 118.7 was first eluted. Replacing the (-CH) in medazepam by (-C=O) the retention time increased as in diazepam 131.9. If R1 is (-H) as in nordazepam, the retention time is also increased (136.5). In oxazepam (125.4) there is an hydroxyl group (-R2). Comparing with nordazepam the retention time is decreased. Also in oxazepam if the (-H) in (-R3) is replaced with (-C1) the retention time increased as in lorazepam (130.3). Substituting (-C1) by (-NO) in (-R4) the retention time increased (nordazepam 136.5, nitrazepam 154.1). Comparing nitrazepam (-R3, -H) with clonazepam (-R3, -Cl) the retention time of the later is higher (160.7).

Table 2
Separation of some drugs of abuse by GLC and HPLC

	HPLC (Minutes)	GLC (RRT)++
Theobromine	3.17	94.5
Benzamphetamine	3.33	89.7
Theophylline	3.33	103.7
Iproniazid	3.35	68.6
Phenobarbital	3.68	100.0
Glutethimide	3.92	90.0
Clonazepam	3.93	160.7
Flunitrazepam	4.37	142.9
Flurazepam	4.42	142.9
Carbamazepine	4.45	124.0
Lorazepam	4.83	130.3
Methaqualone	4.93	113.1
Oxazepam	5.17	125.4
Temazepam	5.45	141.7
L-Ephedrine	5.48	49.66
Norephedrine	5.67	44.6
Sosegon	5.83	121.0
Chlordiazepoxide	5.90	(160.7, 137, 141)
Amphetamine	5.90	26.2
Nordazepam	6.00	136.0
Diazepam	6.53	131.9
Prazepam	9.30	144.7
Medazepam	13.93	118.7

$$++\ RRT = \frac{\text{retention time of the drug}}{\text{retention time of phenobarbital}} \times 100$$

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) showed valuable, successful and sensitive method for separation and identification of some drugs of abuse. The retention time of each drug is shown in Table (2).

Using GLC technique, chlordiazepoxide was degradated into 3 peaks (160.7, 137.0, 142.0) as in Table (2). By HPLC technique a good peak of chlordiazepoxide was obtained after 5.9 minutes. The sequence of elution of the examined drugs, using HPLC is completely different than by GLC, (as shown in Table 2), e.g. by GLC technique the relative retention time of medazepam is 118.7 and clonazepam is 160.6 whereas by HPLC the retention time of medazepam is 13.93 minutes and clonazepam is 3.93 minutes.

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