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FOOTNOTE

* *PROCEEDINGS OF ASSOCIATION OF OFFICIAL RACING CHEMISTS are not available to non-members of that Association, and application for details should be made to the Hon. Secretary, A.O.R.C., Dr. MARGARET A. SULLIVAN, Ph.D., VALLEY RACING LABORATORIES, P.O. BOX 5711, PHOENIX, ARIZONA 85010, U.S.A.*

RESEARCH AND IDENTIFICATION OF TRANQUILLIZERS — USE OF RETENTION INDEX:

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ABSTRACT

At the request of the Service des Haras, our laboratory works on the toxicological problems of the sport-horse. These studies have resulted in the setting up of an anti-doping control for equestrian competitions of various types, not only flat racing.

During events, horses must be calm and docile to the riders' order. Frequently, the latter use tranquillizers to try and win events. The analytical method for the research and identification of these compounds is described.

The technique involves successively:

1. alkalisation of the sample — saliva, blood or urine after enzymatic hydrolysis.
2. extraction with diethyl ether — the recovery is 70% to 90% depending upon the drug.
3. determination by gas-liquid chromatography with use of a retention index for qualitative analysis.

We can detect up to fifteen tranquillizers in any one sample, even when present at such low concentrations as found in saliva.

The use of the retention index is a reliable method for qualitative analysis. For example, the method has been used for three years, during which period the retention index of acetylpromazine remained at 3240 ± 7 . The chromatographic analysis was performed on 3% OV-17 at 290° .

The chromatographic analysis has been performed by three columns of different polarity (OV-1; OV-17; SP-2250). If on the three columns, the retention index of one peak is the same as that of the tranquilizer, a further confirmation is made with the use of a thermionic detector specific for nitrogenous drugs.

In conclusion, this method which is sufficiently precise and specific has been used for anti-doping control.

Introduction

The identification of basic drugs, and in particular of tranquillizers and neuroleptics, is already carried out by gas-liquid chromatography (Parker, Fontan *et al.* 1962; Street 1967; Proelss & Lohmann 1971). For qualitative analysis these methods are based on the measurement of adjusted retention time, or of relative retention time. These retention data are not accurate enough to permit efficient screening.

For the normal practice of qualitative analysis, Ettre (1964) proposes the universal adoption of retention indices (or Kowats indices) as a system of presentation of retention data.

The use of retention indices initially intended for the identification of aliphatic (Kowats 1958) and aromatic compounds (Werli, Kowats 1959) has been extended to other substances: amphetamine (Caddy, Fish 1973; Stead, Moffat *et al.* 1973), some tranquillizers (Moffat, Stead *et al.* 1974).

The literature contains little information on retention indices for the main tranquillizers susceptible of being used for doping in equestrian sports.

We put forward here, essentially in the table, with a short technical explanation, the values obtained in the laboratory for fifteen tranquillizers and neuroleptics, representing many of the commonly used pharmaceutical products.

I Material and Methods

1.1 Gas Chromatography

Packard-Becker Model 417 gas chromatographs were used with a flame ionization detector and 2 metre glass columns. The stationary phases (OV-17, OV-1, SP-2250) were coated (3%) on "Supelcoport" 100-120 mesh (From Supelco, Bellefonte, Pa. U.S.A.).

1.2 Choice of Tranquillizers

The tranquillizers were chosen among the principal representatives of the series of phenothiazines, benzodiazepines, and butyrophenones.

1.3 Preparation of Samples

The tranquillizers were dissolved in twice-distilled ethanol to obtain a concentration of 50 ng/μl solutions.

The tranquillizers were extracted by ether from the biological samples after alkanization of the sample. The details of this technique are described, for the determination of acetylpromazine, by Courtot (1974).

For measuring the retention indices the paraffin solution is mixed with the extract or with standard solutions before the chromatographic analysis.

1.4 Rapid Method of Calculating Retention Index

In the original definition, the retention times of normal paraffins with even carbon atoms were used as reference values, and the retention index (I) of a particular substance was calculated according to the equation:

$$I = 200 \frac{\log V_N (\text{substance}) - \log V_N (PZ)}{\log V_N (PZ + 2) - \log V_N (PZ)} \pm 100Z$$

where

V_N = net retention volume
 PZ = n paraffin with z carbon atoms
 $PZ+2$ = n paraffin with z + 2 atoms

and

Z = an even number; by definition
 $V_N(PZ) \leq V_N (\text{substance}) \leq V_N (PZ+2)$

This method of calculating rapidly becomes fastidious if it is applied to quantitative analysis of a chromatogram obtained from a biological extract. For purposes of speed and simplification, the retention indices are calculated from the measurement of the retention time reduced with the help of an HP65 mini-computer. The calculation programme is drawn from the Hewlett-Packard programme library (STAT-PACK I): logarithmic regression in which Napierian logarithms are replaced by decimal logarithms.

The equation of the regression line is calculated from the reduced retention time of the paraffins and recorded in the memory of the computer. Thanks to this programme, the direct introduction into the mini-computer of the reduced retention times of each compound makes it possible to obtain its retention index directly.

1.5 Determining the Retention Indices on Columns of Different Polarities and at Different Temperatures

Solutions of tranquillizers and n-paraffins were chromatographed on:

two OV-17 columns at 290°
three OV-17 columns at 280°
one OV-17 column at 260°
three OV-1 columns at 260°
three OV-1 columns at 240°
two SP-2250 columns at 250°

The whole analytical process was repeated several times for each drug, and on each occasion the retention index was calculated.

Results and Discussion

This study enables us to establish that:

— For a given column the retention index of fifteen tranquillizers varied by not more than 20 units, while the standard deviation never exceeded 0.2%. Measuring error for the adjusted retention time certainly explains this low variation, since there is no variation in the chromatographic conditions.

— The average retention indices calculated for all the values obtained for a given column vary little from one column to another — this variation is similar to that observed for a given column.

— The temperature changes the values of the retention indices. Whatever the polarity of the column this variation is of the order of 1 unit per °C. It is however difficult to lay down a precise ruling for this.

The validity of this technique of qualitative analysis is confirmed by the overall retention index results. In fixed chromatographic conditions, the retention indices can be considered as physical dimensions characterising a compound. In these conditions the values obtained can be compared from one laboratory to another.

With this in mind, Table I summarizes the average values of all our results. The identification of 15 tranquillizers is thus possible for a laboratory which does not possess the corresponding standards, but only 5 n-paraffins (C24, C28, C30, C32, C36).

Conclusion

The use of retention indices makes it possible to identify, rapidly and accurately, 15 tranquillizers among the most commonly used in equestrian sports.

TABLE I
Retention indices of tranquillizers on OV-17 at 290°, 265°
OV-1 at 260°, 240°, SP-2250 at 250°

Compound	OV-17						OV-1				SP-2250	
	290°		280°		265°		260°		240°		250°	
	n	I	n	I	n	I	n	I	n	I	n	I
promethazine	9	2747	15	2745	15	2724	28	2323	24	2302	7	2672
oxazepam	5	2902	9	2879	8	2855	1	2370	5	2373	1	2659
chlormpromazine	25	2987	27	2971	9	2944	26	2538	25	2515	6	2901
leromepromazine	22	3010	24	2994	12	2976	28	2563	23	2543	9	2928
diazepam	27	3009	23	2998	8	2979	29	2474	24	2439	9	2979
chlordiazepoxide	23	{ 3142 3573	16	{ 3128 3404			26	2527	5	2515	8	3073
chlorazepate	18	3048	7	3129			19	2539			7	3113
cyamepromazine									10	2604		
trifluoperazine	11	3103	12	3089	10	3061	30	2707	26	2682	6	3025
acetylpromazine	27	3253	32	3240	11	3203	29	2735	28	2713	6	3169
azaperone	23	3255	25	3237	6	3200	27	2727	26	2703	7	3170
propionylpromazine	24	3322	25	3303	6	3275	1	2804	17	2789	1	3243
haloperidol	27	3506	26	3486			27	2950	15	2916	4	3417
thioridazine	24	3702	16	3683			25	3104	15	3070	3	3683
droperidol	1	3935					1	3376				

n = number of determinations.

This identification is carried out on three columns of different polarities, and at different temperatures, to ensure the validity of the analysis.

Subsequent to this screening, suspect samples are analysed again using a thermionic detector specifically used for nitrogen compounds, and a flame photometric

detector for sulphur compounds; and then by mass fragmentometry.

This analytical method can be extended to other categories of doping agents permitting their detection and identification even for very low concentrations.

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DISCUSSION

HOUGHTON: Have you tried using linear retention indices at all? Whilst working in Australia I found they used it all the time and found it simple and useful to calculate.

COURTOT: We use this system when there is a main peak on the chromatogram, we use five paraffins and we can calculate indices automatically for all the peaks.

DONIKE: Elution volume is dependent on the laboratory. If the value of retention indices as a critical parameter is to remain you must calculate it as does Courtot. Houghton's method is only an approximation.