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DETERMINATION OF SOME TRICYCLIC NEUROLEPTICS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET AND POLAROGRAPHIC DETECTION

VĚRA PACÁKOVÁ* and KAREL ŠTULÍK
Department of Analytical Chemistry, Charles University, Albertov 2030, 128 40 Prague 2 (Czechoslovakia)
and
HANA TOMKOVÁ
Research Institute for Pharmacy and Biochemistry, Kouřimská 17, 130 00 Prague 3 (Czechoslovakia)
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SUMMARY

The liquid chromatographic behaviour of various neuroleptics from the classes of tricyclic dibenzothiepins, -oxepins, -selenepins and similar substances was studied in an ion-pair reversed-phase system using a C4180 chemically bonded stationary phase and a methanol–water–acetic acid mobile phase. The best separation was attained with 70% methanol, 1.8% acetic acid and water, containing 0.005 *M* sodium pentanesulphonate, 1.2×10^{-4} *M* EDTA and 0.5 g/l Na_2SO_4. For the detection, an ultraviolet spectrophotometric and a polarographic detector were connected in series. The detection was carried out at 254 nm and at a potential of a dropping mercury electrode of −1.0 V (*vs.* Ag/AgCl). The UV detector is universal, while the polarographic detector does not respond to compounds simultaneously containing two heteroatoms (O, S) in the central seven-membered ring. The detection limits are of the order of one to tens of nanograms, the polarographic detector being somewhat more sensitive for most of the compounds; the error of the determination is a few per cent. The method was applied to the determination of isofloxythepin and oxyprothepin in pharmaceutical preparations.

INTRODUCTION

Compounds derived from perathiepin, *i.e.*, 10-(4-methylpiperazino)-10,11-dihydrodibenzo[*b,f*]thiepin, are important neuroleptics. The present paper deals with some compounds of this group (see Table I) that have been synthesized in the Research Institute for Pharmacy and Biochemistry, Prague (see, *e.g.*, refs. 1 and 2). So far, no analytical method has been published for these compounds, except for a patent describing a titrimetric determination[3] and a paper[4] dealing with their gas chromatographic (GC) behaviour. As these substances are almost involatile and some of them, *e.g.*, the –COOH and –OH derivatives, highly polar, their GC analysis requires the use of high working temperatures and, in some cases, derivatization[5]. For

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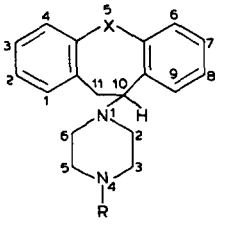
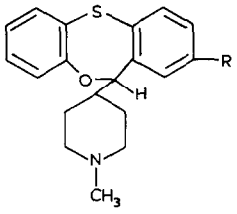
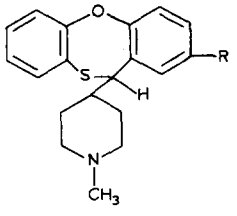
TABLE I

THE COMPOUNDS STUDIED

Parent substance	*Group*	*Substituents*	*Trivial name*	*Code*
	I	X = CH_2, R = CH_3,	–	I-1
	II	X = S, R = CH_3,	Perathiepin	II-1
		8-F	–	II-2
		8-Cl	Octoclothepin	II-3
		8-Br	–	II-4
		8-CH_3	–	II-5
		8-C_2H_5	–	II-6
		8-OCH_3	–	II-7
		8-SCH_3	Methiothepin	II-8
		8-NO_2	–	II-9
		8-NH_2	–	II-10
		8-COOH	–	II-11
		8-CF_3	–	II-12
		8-OC_2H_5	–	II-13
		6-Cl	–	II-14
	III	X = S, R = NH_2	–	III-1
	IV	X = S, R = CH_2CH_2OH,	–	IV-1
		3-F, 8-isopropyl	Isofloxythepin	IV-2
	V	X = S, R = CH_2CH_2OH, 8-SCH_3	Oxyprothepin	V-1
	VI	X = S, R = $CH_2CH_2CH_2OCOCH_3$	–	VI-1
	VII	X = S, R = CH_3,10,11-dehydro,6,8-dichloro	–	VII-1
	VIII	X = O, R = CH_3	–	VIII-1
		8-CH_3	–	VIII-2
		8-Cl	–	VIII-3
		8-SCH_3	–	VIII-4
	IX	X = Se, R = CH_3	–	IX-1
	X	X = $Si(CH_3)_2$, R = CH_3	–	X-1
	XI	R = OCH_3	–	XI-1
		R = Cl	–	XI-2
		R = CF_3	–	XI-3
	XII	R = Cl	–	XII-1
		R = CF_3	–	XII-2

this reason high-performance liquid chromatography (HPLC) is advantageous for their determination[6].

In the present study the HPLC analysis of these compounds was investigated using UV spectrophotometric detection that can be used for all the studied tricyclic derivatives and electrochemical detection which is highly sensitive and selective (see, *e.g.*, ref. 7).

EXPERIMENTAL

Apparatus

An LC-XP liquid chromatograph with a Partisil ODS column (25 × 0.3 cm I.D.), 10 μm (Pye Unicam, Cambridge, U.K.), was used. The samples were injected through a 20-μl loop.

For the detection, an LC-UV variable-wavelength spectrophotometric detector (Pye Unicam) and two electrochemical detectors were used. The UV detector was operated at 254 nm. The voltammetric detector was of the wall-jet type with a glassy carbon working electrode (VDLC), and the polarographic detector employed a macroscopic dropping mercury electrode onto which the eluate was fed horizontally (EDLC). The two electrochemical detectors were manufactured by Laboratorní Přístroje (Prague, Czechoslovakia) and their detailed description can be found in the literature[8,9]. The voltammetric detector was operated in the damped d.c. mode at a potential of +1.4 V and the polarographic detector in the current-sampled d.c. mode with a mercury drop time of 2 sec at a potential of −1.0 V. All the potentials are with respect to a saturated silver chloride electrode at laboratory temperature. The three detectors were connected in series, first the UV, followed by the voltammetric and then the polarographic detector. They were connected by stainless-steel capillary (15 cm × 0.2 mm I.D.).

The polarograms and voltammograms were obtained with a dropping mercury electrode (DME) and a rotating disk glassy carbon electrode (RDE) in the mobile phase, using a PA-3 polarographic analyzer (Laboratorní Přístroje). The UV absorption spectra of the compounds dissolved in the mobile phase were obtained on a Unicam SP-800 spectrophotometer in 1-cm quartz cuvettes.

All solutions were deaerated by passage of helium.

Chemicals

The compounds were kindly provided by Drs. M. Protiva, J. O. Jílek and K. Šindelář of the Research Institute for Pharmacy and Biochemistry, Prague. The pharmaceuticals containing isofloxythepin and oxyprothepin were supplied by the same Institute. Standard solutions (10^{-3} mol/l) of the compounds in the mobile phase were always prepared immediately before the measurements and appropriately diluted.

The mobile phase contained 55–70% (v/v) methanol, 1.8% acetic acid, 0.005 *M* sodium pentanesulphonate, 0.5 g/l $Na_2SO_4 \cdot H_2O$ and $1.2 \cdot 10^{-4}$ *M* EDTA; the rest was water. It was continuously deaerated by passage of helium. All the measurements were performed at laboratory temperature, at a mobile phase flow-rate of 1 ml/min.

Analysis of pharmaceuticals

Twenty pills were finely pulverized and suspended in 250 ml of the mobile phase. The suspension was stirred for 15 min at laboratory temperature, the solid was allowed to settle and 20 μl of the supernatant were injected onto the column. The standard solutions were prepared by dissolving *ca.* 6 mg of the pure active substances (accurately weighed) in the mobile phase in 10-ml standard flasks.

RESULTS AND DISCUSSION

The spectrophotometric, electrochemical and HPLC behaviour of 33 dibenzo[*b,f*]-thiepins, -oxepins and other tricyclic pharmaceutically important compounds was studied;the substances are listed in Table I.

The absorption spectra from 190 to 450 nm were obtained for 10^{-4}–10^{-5} M solutions of the compounds. The λ_{max} values are given in Table II and a typical spectrum (for compound IV-1) is depicted in Fig. 1. Most of the compounds exhibit a sharper absorption maximum at λ = 233–236 nm and a flatter maximum at λ = 253–260 nm. Some also exhibited another maximum at λ = 272–278 nm. Except for the nitro derivative, II-9, that exhibits another large maximum at 340 nm, the UV spectra are not sufficiently characteristic to permit differentiation among the derivatives. For HPLC detection, the first maximum at 233 nm can be used, or that at 254–256 nm which is more advantageous because the absorbance is less dependent on the wavelength and this region is utilized in the most common UV detectors.

The electrochemical behaviour of these compounds was studied, both as regards their oxidation at a glassy carbon rotating disk electrode within a potential region from −0.4 to +1.5 V and their reduction at a dropping mercury electrode at potentials from +0.1 to −1.5 V. With the exception of compounds II-10 and III-1

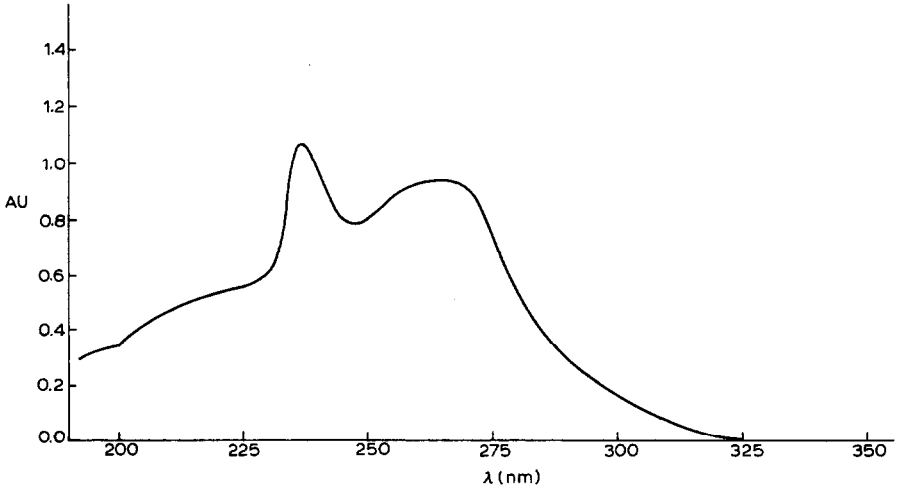


Fig. 1. The UV absorption spectrum of compound IV-1 in 1-cm quartz cuvette; concentration, 1.18 mg per 250 ml mobile phase (70% methanol, 1.8% acetic acid, 0.005 M sodium pentanesulphonate, 0.5 g/l $Na_2SO_4 \cdot H_2O$, $1.2 \cdot 10^{-4}$ M EDTA, water).

which contain an amino group and yield oxidation voltammetric waves with half-wave potentials around +1.2 V, the compounds cannot be oxidized in the studied potential region and thus they cannot be detected by voltammetric detectors. However, most yield reduction polarographic waves with half-wave potentials around −1.0 V. A typical reduction wave is given in Fig. 2, and the half-wave potentials are listed in Table II.

TABLE II

UV SPECTROPHOTOMETRIC, ELECTROCHEMICAL AND CHROMATOGRAPHIC CHARACTERISTICS OF THE STUDIED COMPOUNDS

Code	λ_{max} *(nm)*	$E_{1/2(DME)}$ *(V)*	*k'*		*Detection limit (ng)*	
			60% methanol	*70% methanol*	*UV*	*polarogr.*
Thioxanthene	235, 250, 265*	–	–	1.30	29	–
I-1	235	−0.95	–	1.62	95	19
II-1	233	−0.96	4.01	2.24	18	9
II-2	233	−1.05	3.46	1.86	15	13
II-3	236, 265*	−0.98	–	2.62	15	19
II-4	233, 258–268**	−1.01	5.33	2.60	42	15
II-5	233	−0.96	5.39	2.71	32	19
II-6	233–253*	–***,§	6.60	3.11	21	14
II-7	–§	–§	4.40	2.09	–	–
II-8	233, 275*	−1.03	5.77	3.03	19	14
II-9	233, 340*	−0.40	3.14	1.66	26	18
II-10	233, 260*	–***,§§	2.04	1.79	8	21
II-11	233, 257–267**	−0.93	3.23	1.54	19	15
II-12	233, 273§§§	−1.07	4.83	2.11	20	30
II-13	233; 253	–***	5.35	2.85	20	12
II-14	233, 257–267**	−0.95	–	3.08	52	9
III-1	233, 253**	−0.93	1.22	0.58	16	17
IV-1	233, 257–267**	−0.95	3.14	1.50	11	7
IV-2	235, 252, 275	–***	–	2.65	10	2
V-1	236, 277*	–***	–	1.66	9	4
VI-1	233	−0.98	2.19	1.02	54	10
VII-1	233, 272*	–***	–	4.20	32	16
VIII-1	233, 270**	−0.95	2.13	1.45	11	9
VIII-2	233, 257§§§	−0.86	3.63	1.80	31	7
VIII-3	233, 272§§§	−0.97	3.37	1.87	29	18
VIII-4	233, 257*	−0.94	3.58	2.04	7	5
IX-1	235, 276**	−0.93	–	2.71	25	8
X-1	235	−0.97	–	2.58	28	15
XI-1	233*, 278*	−1.00	3.92	2.31	14	25
XI-2	233, 254, 273	–	4.27	2.15	11	–
XI-3	233, 253**, 282**	–***	3.92	1.82	16	50
XII-1	233, 254	−1.03	2.85	1.47	8	129
XII-2	253	–	2.97	1.37	12	–

* High peak.
** Flat peak.
*** Shift in H_2 evolution.
§ Pure substance was not available.
§§ Non-reducible, but anodic wave at +1.2 V (glassy carbon).
§§§ Low peak.

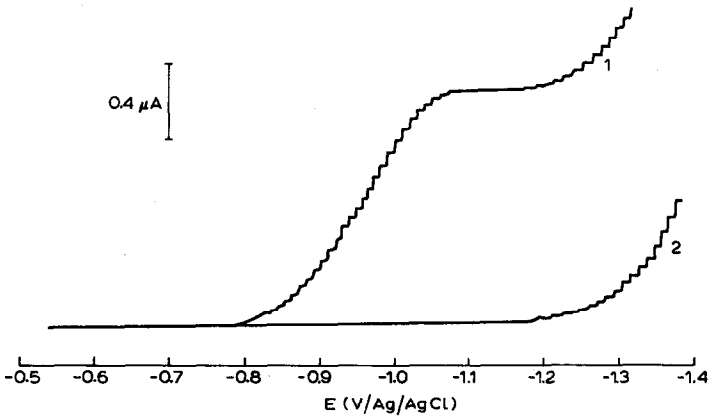


Fig. 2. The polarographic curve of compound IV-1; concentration, 14.1 mg per 50 ml mobile phase (mobile phase composition as in Fig. 1). Curves: 1, compound IV-1; 2, base electrolyte.

The height of these polarographic waves is proportional to the concentration and logarithmic analysis of the waves yields a linear dependance of log $[I/(I_1-I)]$ *vs.* E, the reciprocal of the slope being about 0.08 V. These dependences suggest that the limiting currents are controlled by diffusion and that the charge-transfer reaction is irreversible. Some of the compounds (II-6, II-10, II-13, IV-2, VII-1, XI-3) do not yield a reduction wave, but in their presence the hydrogen evolution shifts to more positive potentials or their waves merge with the hydrogen-ion reduction current and so they can also be detected polarographically. Only XI-2, XII-2 and thioxanthene do not yield polarographic waves and do not cause a shift in the hydrogen evolution potential; thus they cannot be detected polarographically. The polarographic detection is least sensitive for the compounds containing two heteroatoms (O, S) in the central seven-membered ring.

Thus, it follows from the electrochemical study that voltammetric detection of these compounds at a solid electrode is virtually impossible. For polarographic detection, potentials more negative than -1.2 V seem to be most suitable. However, at potentials more negative than *ca.* -1.0 V the detector residual current rapidly increases and therefore a potential of -1.0 V was chosen. The polarographic detection is very sensitive and reproducible at this potential, even when this value corresponds to the rising part of the polarographic waves. In flowing solution and the very limited space of the cell, the polarographic waves apparently shift to somewhat more positive potentials compared with quiescent solution due to enhanced mass transport to the electrode, and thus the limiting currents are attained even at -1.0 V. The detection of the compounds that do not yield well developed reduction waves is then based on the difference in the current in the presence and absence of the solute at a potential of -1.0 V. As the exponential increase in the current is very steep in this case, the detection is also very sensitive (see below). The reproducibility of measurement is similar to that obtained with the compounds which yield well developed waves. In general, the reproducibility of the results is better for substances exhibiting lower detection limits (see *e.g.*, Table III).

TABLE III

DETERMINATION OF OXYPROTHEPIN AND ISOFLOXYTHEPIN IN PILLS

	Theor. amount per pill (ng)	*Found*			
		UV		*Polarogr.*	
		Amount per pill (ng)	*S.D. (%)*	*Amount per pill (ng)*	*S.D. (%)*
Oxyprothepin	5	5.41	2.6	5.38	2.6
Isofloxythepin	4	4.43	4.7	4.40	0.8

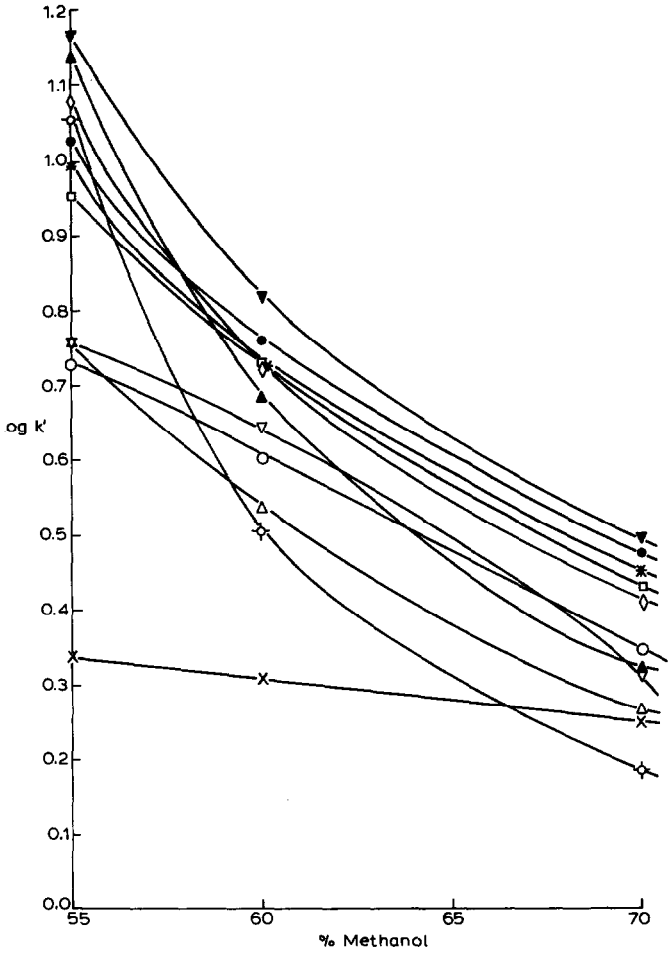


Fig. 3. Dependence of log k' on the mobile phase composition. Compounds: II-6 (▼; II-12 (▲); II-4 (◇); II-11 (⊖); II-8 (●); II-3 (×); II-13 (★); II-5 (□); II-2 (△); II-7 (▽); II-1 (○).

Chromatographic behaviour

On the Partisil ODS non-polar stationary phase the capacity factors of the solutes were measured at various mobile phase compositions and are given in Table II. The mobile phase was an aqueous methanol solution, containing sodium pentanesulphonate as the ion-pairing agent and acetic acid to enhance the dissociation of the basic tricyclic substances. EDTA was added to complex any metal ions present and sodium sulphate increased the electrical conductivity of the mobile phase. An increase in the methanol concentration markedly decreases the capacity factors. The dependence of log k' on the methanol percentage for selected perathiepin derivatives (those substituted in position 8) is given in Fig. 3. The optimum methanol content is 70%. An example of the separation of a mixture of the studied compounds using UV and polarographic detection is shown in Fig. 4. It is seen that the peaks are sufficiently sharp and symmetrical and the longest retention time is 17 min.

The same effect as that caused by increasing the methanol concentration can be attained by increasing the temperature. In reversed-phase chromatography with water–methanol as mobile phase, enthalpy–entropy compensation occurs (see ref. 10). The typical linear relationships, between the logarithm of the capacity factor and reciprocal temperature is shown in Fig. 5 for perathiepin derivatives. Except for compounds II-3, II-8 and II-13, the resolution is unaffected by a change in the temperature over the studied range and the only favourable effect of an increased temperature is a shorter analysis time. The temperature dependence of the capacity factors for tricyclic compounds was studied in greater detail previously[6].

The detection limits for the UV and polarographic detection were determined

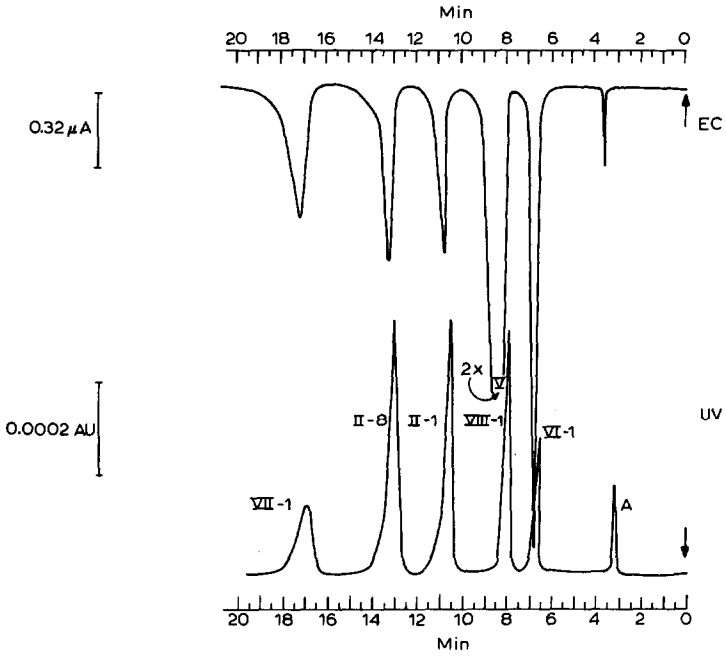


Fig. 4. A typical chromatogram of the studied compounds with simultaneous UV and polarographic detection. Stationary phase: Partisil ODS. Mobile phase as in Fig. 1; flow-rate 1 ml/min. Substances: VI-1. 0.48 μg; VIII-1, 0.37 μg; II-1, 0.30 μg; II-8, 0.25 μg; VII-1, 0.23 μg. A = Dead time.

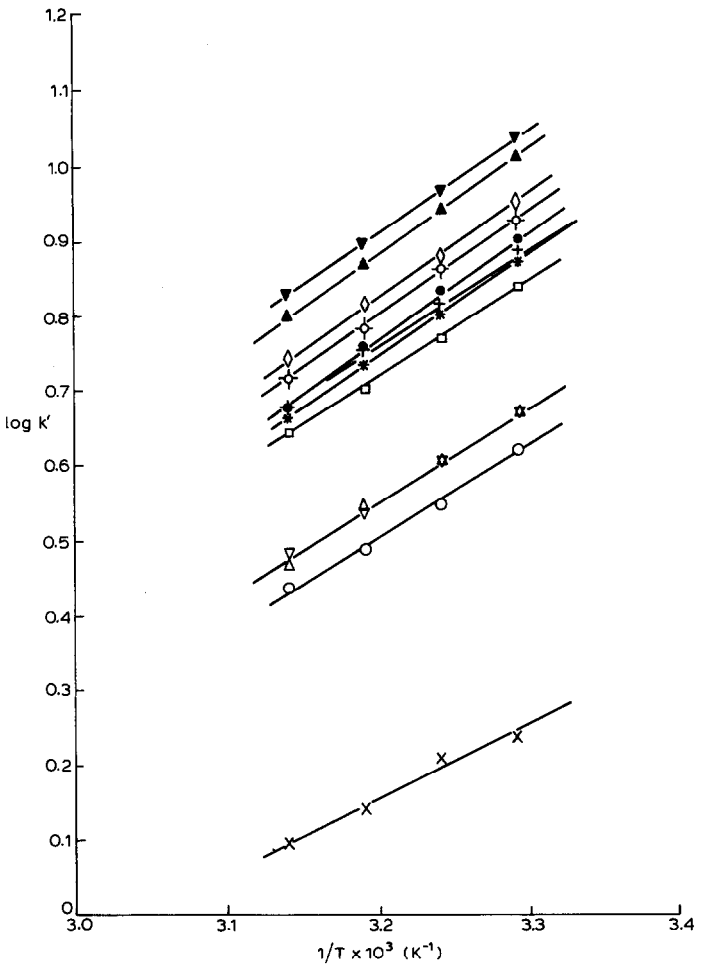


Fig. 5. Dependence of log k' on the reciprocal column temperature. For compounds see Fig. 3.

using the optimum mobile phase composition and are given in Table II, from which it follows that polarographic detection is more sensitive than UV detection for most of the compounds, especially for the pharmaceuticals isofloxythepin and oxyprothepin (detection limits, 2 and 4 ng, respectively). For this reason this method was applied to the determination of the active substances in pills of these pharmaceuticals. The procedure is given in Experimental; the absolute calibration method was used. Each measurement was repeated at least five times and the average values are given in Table III. It is evident that the agreement in the determined amounts of the substances is good for the two detectors used. As polarographic detection is more sensitive, its precision is better than that of the UV detector. The high sensitivity and especially the selectivity of polarographic detection is very promising for the study of the kinetics of these substances in body fluids.

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