HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF THIOBENZAMIDE DERIVATIVES WITH ULTRAVIOLET PHOTOMETRIC AND ELECTROCHEMICAL DETECTION*

MARTINA ŘÍHOVÁ, VĚRA PACÁKOVÁ and KAREL ŠTULÍK*

Department of Analytical Chemistry, Charles University, Albertov 2030, 128 40 Prague 2 (Czechoslovakia)

and

KAREL WAISSER

Faculty of Pharmacy, Charles University, Heyrovského 1203, 501 65 Hradec Králové (Czechoslovakia)

(First received January 27th, 1986; revised manuscript received February 21st, 1986)

SUMMARY

Twenty-five thiobenzamide derivatives were separated on a reversed-phase system on various stationary phases, with UV photometric and voltammetric detection. The best results were obtained using a Partisil ODS column (10 μm, 250 mm $\times$ 4.6 mm I.D.) with a mobile phase consisting of 0.05 M sodium dihydrogen phosphate with 30% (v/v) methanol, containing $1.2 \cdot 10^{-4}$ M EDTA, at a flow-rate of 1.0 ml/min. Voltammetric detection on a carbon-fibre electrode at $+1.4$ V (silver–silver chloride) is more sensitive than UV photometric detection at 254 nm, typical detection limits being *ca.* 0.1 and 1.0 ng. Voltammetric calibration curves exhibit good linearity and the measurements are reproducible (relative standard deviation of *ca.* 2%).

INTRODUCTION

Thiobenzamide derivatives have been studied by many workers (for recent examples, see refs. 1–4) because of their antituberculotic activity. Many new substances have been synthesized and their physico-chemical properties investigated[1,5] (*e.g.* the UV absorption and vibrational spectra, the dissociation constants, dipole moments, electrochemistry, solubility in various solvents, etc.). The effect of various aromatic ring substituents on the biological activity has been studied, and the biological activity has been correlated with the physico-chemical properties of the substances (see refs. 6 and 7, and references therein).

So far, little attention has been paid to the chromatographic behaviour of these substances; a thin-layer separation has been described[8]. This paper deals with the high-performance liquid chromatography (HPLC) of selected thiobenzamide deriv-

---

* Part 11 in the series: Physical and Physico-chemical Properties of Thiobenzamides. For part 10 see ref. 1.

TABLE I

SURVEY OF THE THIOBENZAMIDE DERIVATIVES STUDIED

$\langle\bigcirc\rangle$— $CSNH_2$
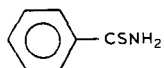
Thiobenzamide.

No.	Name	Dipole moment, $\mu^\star$	$pK_a^{\star\star}$
1	Thiobenzamide (TBA)	15.8	11.55
2	2-Nitrothiobenzamide (2-NO$_2$-TBA)		
3	3-Nitrothiobenzamide (3-NO$_2$-TBA)	15.4	10.55
4	4-Nitrothiobenzamide (4-NO$_2$-TBA)	18.1	10.55
5	3-Methoxythiobenzamide (3-CH$_3$O-TBA)		
6	4-Methoxythiobenzamide (4-CH$_3$O-TBA)		11.85
7	3-Methylthiobenzamide (3-CH$_3$-TBA)	16.3	11.55
8	4-Methylthiobenzamide (4-CH$_3$-TBA)	16.1	11.70
9	2-Hydroxythiobenzamide (2-OH-TBA)		
10	3-Hydroxythiobenzamide (3-OH-TBA)		
11	4-Hydroxythiobenzamide (4-OH-TBA)		
12	2-Chlorothiobenzamide (2-Cl-TBA)		
13	3-Chlorothiobenzamide (3-Cl-TBA)	15.0	11.10
14	4-Chlorothiobenzamide (4-Cl-TBA)	14.8	11.35
15	3-Bromothiobenzamide (3-Br-TBA)		
16	4-Bromothiobenzamide (4-Br-TBA)		11.35
17	4-Trifluoromethyl-thiobenzamide (4-CF$_3$-TBA)		
18	4-Methylthiothiobenzamide (4-SCH$_3$-TBA)		
19	4-Dimethylaminothiobenzamide (4-N(CH$_3$)$_2$-TBA)		
20	4-Thioamidethiobenzamide (4-CSNH$_2$-TBA)		
21	3,4-Dichlorothiobenzamide (3,4-Cl$_2$-TBA)		
22	3,5-Dichlorothiobenzamide (3,5-Cl$_2$-TBA)		
23	3,5-Dibromothiobenzamide (3,5-Br$_2$-TBA)		
24	2-Amino-5-nitrothiobenzamide (2-NH$_2$-5-NO$_2$-TBA)		
25	2-Amino-3-chloro-5-nitrothiobenza-mide (2-NH$_2$-3-Cl-5-NO$_2$-TBA)		

$\star$ In 10^{-30} C m units; taken from ref. 5.
$\star\star$ Taken from ref. 1.

atives on various chemically bonded phases, using UV photometric and electrochemical detection. The correlation of the chromatographic retention data with the structure of the test substances will be discussed in another paper.

EXPERIMENTAL

The thiobenzamide derivatives were synthesized at the Faculty of Pharmacy, Charles University, Hradec Králové, Czechoslovakia, and are listed in Table I. The p.a. chemicals employed for the preparation of the mobile phases were obtained from Lachema (Czechoslovakia) and from Merck (F.R.G.) and were not further purified.

The chromatographic measurements were carried out on an LC-XP instrument with an LC-UV variable-wavelength UV photometric detector (Pye Unicam, U.K.) and a carbon-fibre voltammetric detector of our own construction[9] with and EDLC measuring system (Laboratorní Přístroje, Czechoslovakia), using a silver–silver chloride reference and a platinum counter electrode. The two detectors were connected in series, the UV photometric detector first using a shortest possible stainless steel capillary (0.2 mm I.D.).

The spectrophotometric measurements were performed on an SP-800 instrument (Unicam, U.K.) with 1-cm quartz cuvettes. The polarization curves of the test substances were obtained using a glassy carbon rotating-disk electrode (3 mm in diameter) and a PA-3 polarographic analyser (Laboratorní Přístroje), at a potential scan-rate of 5 mV/s.

The chromatographic columns used are listed in Table II. On the basis of preliminary experiments, a mobile phase consisting of aqueous 0.05 M sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 12H_2O$) mixed with methanol was used. The disodium salt of ethylenediaminetetraacetic acid (EDTA) was added to the mobile phase to obtain a concentration of $1.2 \cdot 10^{-4}$ M, in order to mask trace metal ions. The pH of the mobile phase was adjusted with phosphoric acid or sodium hydroxide. The mobile phase was filtered before use and degassed by passage of helium. The mobile phase flow-rate was 0.5 ml/min for the 150-mm columns and 1.0 ml/min for the 250-mm columns.

TABLE II

CHARACTERISTICS OF THE COLUMNS AND THE OPTIMAL COMPOSITIONS OF THE MOBILE PHASE

No.	Packing	$\bar{d}_p$ (µm)	Length (mm)	I.D. (mm)	Methanol content (%)	Resolution*		
						$R_{5,6}$	$R_{15,16}$	$R_{14,25}$
1	Separon SI C$_{18}$**	10	150	3.0	40	0.25	0.60	2.00
2	Partisil ODS***	10	250	4.6	30	1.96	0.70	2.57
3	Separon SI CN**	10	250	4.6	5	1.25	0.60	0.32
4	Separon SI CN**	5	150	3.0	2	1.19	0.67	0.25
5	Separon SI Phenyl**	7	150	3.0	25	0.02	3.26	0.79

* The subscripts 5, 6, 14, 15, 16 and 25 designate the substances according to Table I.
** Laboratorní Přístroje, Czechoslovakia.
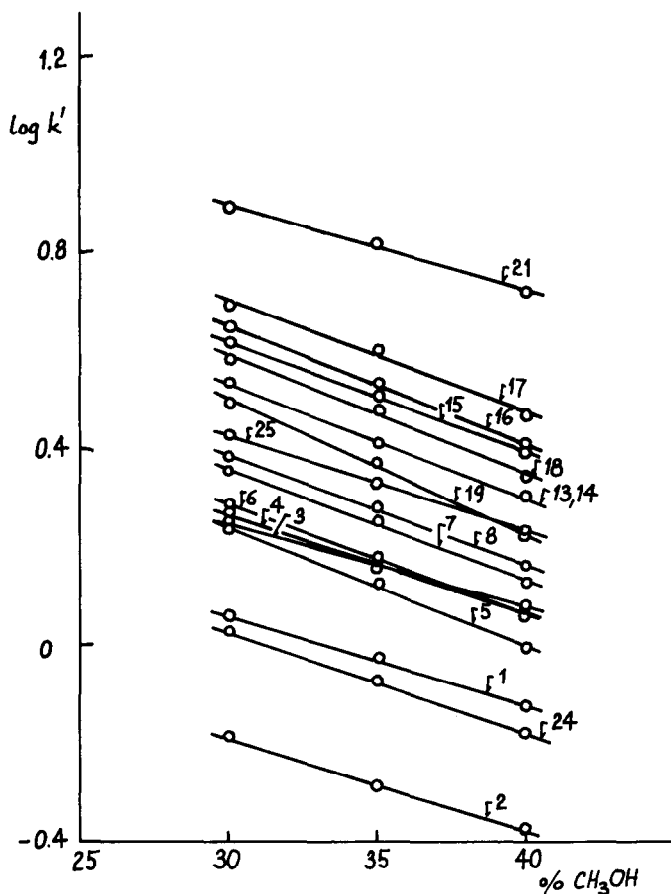*** Pye Unicam, U.K.

Fig. 1. Dependence of the logarithms of the capacity ratios on the methanol content in the mobile phase. Column, Partisil ODS. For the numbering of the substances, see Table I.

Solutions of the test substances were prepared immediately before the measurement by dissolving *ca.* 2 mg of the substance in 10 ml of methanol and diluting appropriately with the mobile phase. For the spectrophotometric and voltammetric measurements, *ca.* 4 mg of the substance were dissolved in 50 ml of methanol–0.05 *M* NaH$_2$PO$_4$ · 12H$_2$O (aq.) (30:70).

All the measurements were carried out at laboratory temperature. The electrode potentials are referred to the saturated silver–silver chloride electrode. The detector responses were treated by linear regression.

RESULTS AND DISCUSSION

Separation

The optimal compositions of the mobile phase for the separation of the 25 thiobenzamide derivatives listed in Table I, obtained by measuring the dependences of the capacity ratios on the methanol content, are given in Table II for various

TABLE III

LOGARITHMS OF CAPACITY RATIOS EXTRAPOLATED TO ZERO CONCENTRATION OF METHANOL

Substance	Column No.				
	1	2	3	4	5
2-NO$_2$-TBA	0.871	0.381	−0.116	0.239	0.530
2-NH$_2$-5-NO$_2$-TBA	1.264	0.641	−0.042	0.178	0.672
TBA	1.454	0.612	0.051	0.365	0.660
4-CH$_3$O-TBA	1.826	1.003	0.335	0.581	1.108
3-CH$_3$O-TBA	1.805	0.926	0.284	0.572	1.108
4-NO$_2$-TBA	1.924	0.881	−0.036	0.259	0.792
4-N(CH$_3$)$_2$-TBA	2.262	1.290	0.638	1.196	1.715
3-CH$_3$-TBA	2.070	1.040	0.360	0.732	1.198
4-CH$_3$-TBA	2.089	1.040	0.383	0.718	1.198
2-NH$_2$-3-Cl-5-NO$_2$-TBA	2.146	1.086	0.301	0.640	1.266
4-SCH$_3$-TBA	2.447	1.325	0.597	0.940	1.683
3-Cl-TBA	2.259	1.192	0.342	0.665	1.307
4-Cl-TBA	2.369	1.217	0.307	0.654	1.147
4-CF$_3$-TBA	.2.821	1.419	0.369	0.729	1.707
3-NO$_2$-TBA	−	0.790	0.100	0.413	0.943
3-Br-TBA	−	1.334	0.474	0.826	1.559
4-Br-TBA	−	1.409	0.455	0.777	1.506
3,4-Cl$_2$-TBA	−	1.759	0.596	0.978	1.907

chemically bonded phases (C$_{18}$, CN, phenyl). The dependences are similar for all the stationary phases and an example (for the Partisil ODS column) is given in Fig. 1. Table II also gives the resolution values for some pairs of substances that are difficult to separate. The capacity ratios extrapolated to zero methanol concentration are summarized in Table III.

It can be seen from Tables II and III that an optimal separation of the test substances is attained on the Partisil ODS column. An example of the separation with electrochemical detection is given in Fig. 2. The *meta* and *para* derivatives are also separated well on the phenyl phase; the CN phase is less selective for the thiobenzamide derivatives.

Except for the capacity ratio of 4-dimethylaminothiobenzamide, which strongly decreases at low pH values, apparently owing to preferential protonation of the N-dimethylamino group, those of the other substances are independent of pH in the range 2.5–6.0. This is not surprising in view of the pK_a values of the substances given in Table I. The pH would have to be increased above 10 to influence the capacity ratios substantially; this is, however, impossible with the present stationary phases, because of degradation of silica gel at high pH values.

The retention behaviour of the C-3 and C-4 derivatives of thiobenzamide can be explained on the basis of the dipole moments of the substances, taken from ref. 5 (Table I). Whereas on a non-polar stationary phase the differences in the dipole moments do not affect the retention characteristics of the substances, the difference in the dipole moments of the 3- and 4-nitro derivatives (15.4 and 18.1, respectively) causes a substantial difference in the capacity ratios on the CN-phase.
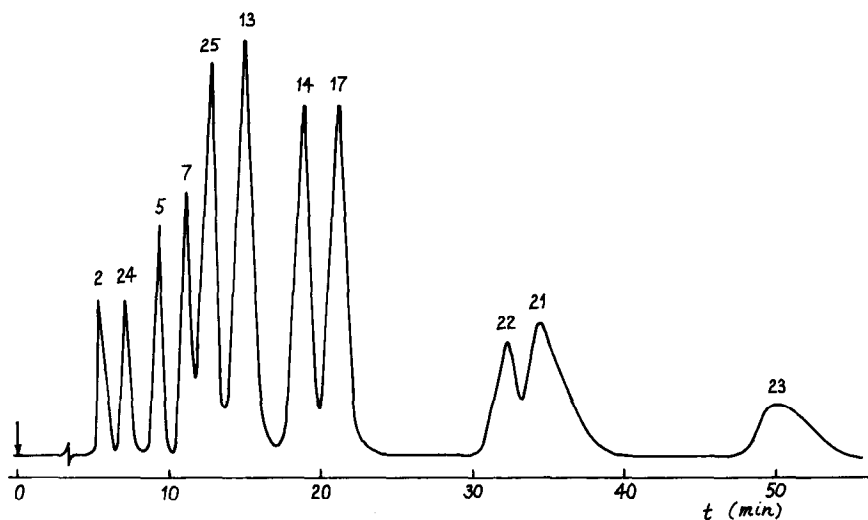
Fig. 2. Chromatogram of eleven thiobenzamide derivatives on the Partisil ODS column. Mobile phase: 0.05 M NaH$_2$PO$_4$ · 12H$_2$O with 30% (v/v) methanol and 1.2 · 10^{-4} M EDTA at a flow-rate of 1.0 ml/min. Electrochemical detection at a potential of +1.4 V. For the numbering of the substances, see Table I.

TABLE IV

UV ABSORPTION MAXIMA AND UV PHOTOMETRIC AND VOLTAMMETRIC DETECTION LIMITS (SIGNAL-TO-NOISE RATIO 2)

| Substance	Absorption maxima, λ_{max} (nm)	Detection limits (ng)	
		UV photometric, 254 nm	Voltammetric, +1.4 V
2-NO$_2$-TBA	215; 263	0.51	0.33
3-NO$_2$-TBA	215; 235	0.51	0.37
4-NO$_2$-TBA	215; 255	0.53	0.37
2-NH$_2$-5-NO$_2$-TBA	220; 290; 360	0.52	0.19
TBA	215; 248; 290	0.40	0.29
3-CH$_3$-TBA	215; 254; 292	0.48	0.24
4-CH$_3$-TBA	209; 259; 292	0.62	0.30
3-CH$_3$O-TBA	220; 255; 295	0.53	0.17
4-CH$_3$O-TBA	215; 260; 300	0.49	0.17
2-Cl-TBA	212; 235; 275	0.47	0.20
3-Cl-TBA	220; 240; 292	0.73	0.22
4-Cl-TBA	216; 253; 290	0.94	0.20
2-OH-TBA	215; 257; 300	0.37	0.09
3-OH-TBA	220; 256; 295	0.50	0.07
4-OH-TBA	218; 275; 302	0.44	0.07
4-SCH$_3$-TBA	215; 300; 320	1.41	0.33
4-N(CH$_3$)$_2$-TBA	215; 235; 295	2.53	0.77
3-Br-TBA	220; 295	0.92	0.34
4-Br-TBA	208; 258; 295	1.17	0.37
3,5-Br$_2$-TBA	218; 298	3.10	0.96
3,4-Cl$_2$-TBA	220; 252; 295	1.71	0.80
3,5-Cl$_2$-TBA	220; 240; 295	2.33	0.57
4-CSNH$_2$-TBA	202; 230; 258; 300	0.45	0.13
4-CF$_3$-TBA	220; 235; 290	1.41	0.31
2-NH$_2$-3-Cl-5-NO$_2$-TBA	215; 280; 360	1.12	0.21
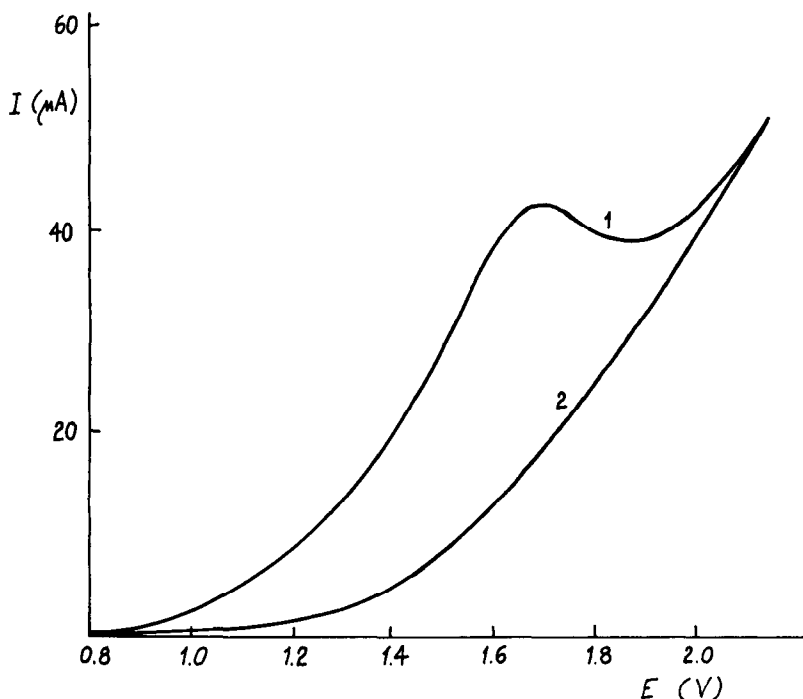
Fig. 3. Voltammograms of (1) 4-CH₃O-TBA in the mobile phase and (2) of the mobile phase alone.

The capacity ratios extrapolated to zero methanol content (Table III) can be used for a prediction of the retention behaviour of other thiobenzamide derivatives, on the basis of additivity of the contributions from individual substituents. For example, the capacity ratio for 3,4-dichlorothiobenzamide on Partisil ODS column can be calculated from the capacity ratios of 3-chloro- and 4-chlorothiobenzamides. The calculated value of k is 1.797 and the experimental value 1.759.

TABLE V

CALIBRATION DATA FOR VOLTAMMETRIC DETECTION AT +1.4 V

Substance	Calibration curve slope (nA/ng)	Correlation coefficient	R.S.D. (%)* s_{xy} (n = 25)
2-NO₂-TBA	17.7 ± 0.3	0.9992	3.9
3-NO₂-TBA	14.4 ± 0.3	0.9984	8.6
3-CH₃-TBA	26.4 ± 0.2	0.9997	4.9
4-CH₃-TBA	21.2 ± 0.4	0.9991	5.7
3-CH₃O-TBA	21.2 ± 0.4	0.9990	4.1
4-CH₃O-TBA	34.2 ± 0.1	1.0000	2.8
TBA	18.6 ± 0.1	0.9997	3.3
2-NH₂-3-Cl-5-NO₂-TBA	22.4 ± 0.1	0.9999	3.9

* Relative standard deviation of the linear regression dependence.

Detection

The UV absorption spectra of all the substances studied have been measured, and the λ_{max} values are summarized in Table IV. Most of the substances absorb around 215, 240–260 and 300 nm. We chose a wavelength of 254 nm for detection, which corresponds to the second absorption maximum and is available with any UV detector. The detection limits, obtained at a signal-to-noise ratio of two, are given in Table IV.

Thiobenzamide derivatives can be oxidized at solid electrodes, and a typical voltammogram is given in Fig. 3. It is evident that the electrode reactions are irreversible and that the reaction products are adsorbed on the electrode surface (a typical drop in the limiting current at *ca.* + 1.8 V). For detection a potential of + 1.4 V has been selected, at which an optimal signal-to-noise ratio is obtained. The detection limits, obtained in the same way as with the UV photometric detector, are given in Table IV, which shows that the voltammetric detector is more sensitive to most of the substances than the UV photometric detector, and that the detection limits are lowest for hydroxy derivatives of TBA, very low for amino derivatives and highest for dihalogeno and N-dimethyl derivatives of TBA. The parameters of the voltammetric calibration curves for selected substances (Table V) demonstrate a very good linearity. The reproducibility of measurement is 2.0–2.3% (relative standard deviation) and the detector noise amounts to *ca.* 10^{-10} A.

CONCLUSION

The substances studied can be satisfactorily separated in a reversed-phase system using a C_{18} stationary phase and a mixture of aqueous phosphate with methanol as the mobile phase. Voltammetric detection is more sensitive than UV photometric detection and exhibits good linearity and reproducibility.

REFERENCES

1 K. Waisser and M. Čeladník, in M. Tichý (Editor) *QSAR in Toxicology and Xenobiochemistry*, Elsevier, Amsterdam, 1985, pp. 339–344.
2 J. K. Seydel, *Antibiot. Chemother.*, 12 (1964) 135.
3 B. Tornetta, F. Guerrera and C. Mazarino, *Bull. Chim. Farm.*, 112 (1973) 822, 832.
4 V. I. Cohen, N. Rist and S. Clavel, *Eur. J. Med. Chem.*, 10 (1975) 134, 137, 140.
5 O. Exner and K. Waisser, *Collect. Czech. Chem. Commun.*, 47 (1982) 828.
6 K. Waisser, M. Čeladník, K. Palát, R. Karlíček, Ž. Odlerová, F. Bartoš and J. Dršata, *Pharmazie*, 38 (1983) 874.
7 K. Waisser, J. Dršata, F. Bartoš and K. Kosař, in M. Tichý (Editor) *QSAR in Toxicology and Xenobiochemistry*, Elsevier, Amsterdam, 1985, pp. 91–96.
8 K. Waisser, H. Synková and M. Čeladník, *Čs. Farm.*, 32 (1983) 3.
9 K. Štulík, V. Pacáková and M. Podolák, *J. Chromatogr.*, 298 (1984) 225.