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Short communication

Reversed-phase high-performance liquid chromatographic method for the quantitative determination of alkylbis(2-benzothiazolylsulfen)amides

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Abstract

An isocratic, reversed-phase HPLC procedure was developed for the simultaneous determination of isopropyl-, *tert.*-butyl-, *tert.*-amyl-, cyclohexylbis(2-benzothiazolylsulfen)amides. Quantitation is performed on a C₁₈ bonded-phase column (Separon SGX C₁₈, 5 μm) using N-dicyclohexyl-2-benzothiazolesulfenamide as internal standard followed by UV photodiode-array detection. The precision ($n=7$) for all derivatives of alkylbis(2-benzothiazolylsulfen)amides is within 1.5%. Identification of the compounds also in the mixtures was done by NMR spectroscopy.

1. Introduction

Alkylbis(2-benzothiazolylsulfen)amides (alkylbisBS) are used in the rubber industry as vulcanisation accelerators. Like the N-alkyl-2-benzothiazolesulfenamides (alkylBS), these compounds are thermolabile. Therefore, HPLC methods can not be used in the analysis of these compounds.

Šmejkal et al. [1] applied a reversed-phase HPLC method with a macroporous gel, Separon SE, for the determination of some compounds in rubber, one of them being N-morpholinyl-2-sulfenamide.

Various LC–MS techniques were studied for their ability to detect and identify minor components in benzothiazole derived compounds [2].

CyclohexylBS, cyclohexylbisBS and 2,2'-dithiobisbenzothiazole alone and in their mixtures were analyzed by Isaeva et al. [3] using coulometric titration in acetic acid with electrically generated bromine and by Borisova et al. [4] using oxidative bromination in acetic acid with amperometric detection.

In this paper, a simple, isocratic reversed-phase HPLC procedure is described for the simultaneous determination of isopropyl-, *tert.*-butyl-, *tert.*-amyl-, and cyclo-hexylbis(2-benzothiazolylsulfen)amides. Quantitation is performed on a C₁₈ bonded-phase column using N-dicyclohexyl-2-benzothiazolesulfenamide as internal standard. Identification of the com-

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pounds also in the mixtures is done by NMR spectroscopy.

2. Experimental

2.1. Materials

Acetonitrile was of HPLC quality (Labscan, Ireland). Water was purified with an osmometric system made by Wilhelm Werner (Germany). Other chemicals were of analytical reagent grade.

The derivatives of alkylbisBS (Fig. 1) were prepared by the method described in Ref. [5]

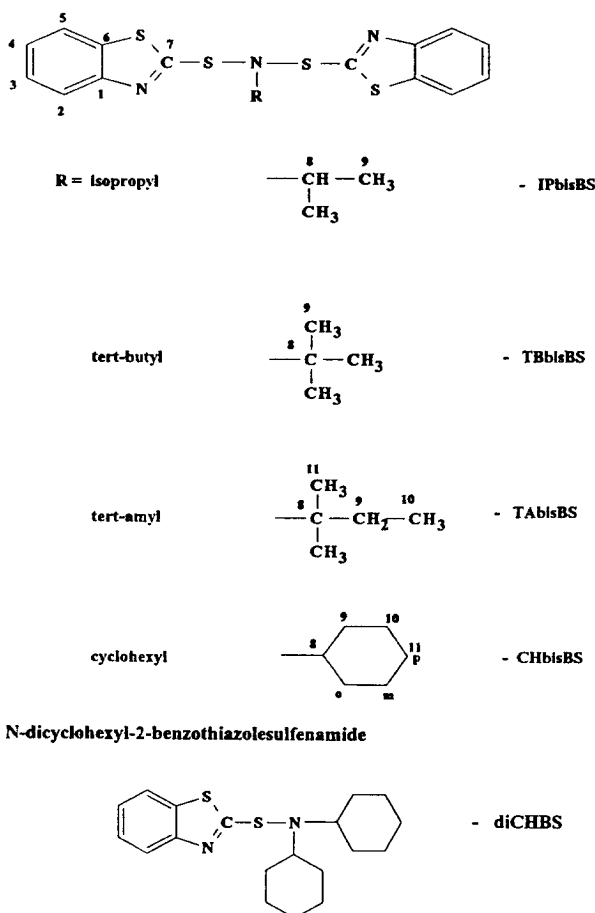


Fig. 1. Derivatives of alkylbis(2-benzothiazolyl)sulfenamides and dicyclohexyl-2-benzothiazolylsulfenamide.

and recrystallized from diethyl ether. Purity was checked by NMR spectroscopy. Melting points of isopropylbisBS, *tert.*-butylbisBS, *tert.*-amylbisBS and cyclohexylbisBS were 106–107°C, 144–146°C, 125–126°C and 133–134°C, respectively. N-Dicyclohexyl-2-benzothiazolylsulfenamide was a commercial product recrystallized from ethanol. Samples containing about 15 µg of the component in 1 cm³ of acetone were prepared freshly before use. A longer than 4-h contact of the alkylbisBS derivatives with acetone causes a decrease of the precision. Solvents such as heptane, hexane and others used to prepare the real samples containing these compounds were removed by vacuum distillation. The mobile phases and the liquid samples were filtered and degassed under vacuum before use. The mobile phases were prepared volumetrically from various amounts of methanol, acetonitrile, tetrahydrofuran and water.

2.2. NMR spectroscopy

Measurements were performed at 30°C using a Varian VXR-300 spectrometer with an operating frequency of 300 MHz for protons and 75 MHz for carbons. Standard [¹H]-, [¹³C]- and [¹³C]-ATP spectra were measured. For the same samples, additional H,H and one-bond or long-range H,C correlation NMR experiments were performed. The samples were dissolved in CHCl₃ (ca. 50 mg/cm³).

2.3. High-performance liquid chromatography

Elution was performed using a Rheodyne injection valve fitted with a 4-µl injection loop (Shimadzu, Kyoto, Japan), an LC 10AD solvent delivery system, a 150 × 3.0 mm I.D. Separon SGX C₁₈ column filled with 7 or 5 µm particle diameter ODS silica (Tessek, Czech Republic), and a Shimadzu SPD M6A photodiode-array detector operating in the range 195–670 nm, equipped with a Syntron computer and Class LC 10 software for control of the system and data acquisition and integration. Another column tested was packed with Nucleosil 120-3C₁₈ 7 µm particle diameter (150 × 3.0 mm I.D.). All analy-

sis were carried out at ambient temperature. The measurements were performed in triplicate. The precision of the determination for all derivatives was calculated from seven replicated injections. The dead time was determined by using water as the non-retained compound. The capacity factor was calculated as $k' = (t_R - t_0)/t_0$, where t_R is the retention time, t_0 is the dead time and the resolution was given by the equation $R_s = (t_{R,j} - t_{R,i})/2(\sigma_i + \sigma_j)$, where $t_{R,j}$ and $t_{R,i}$ are the retention times of compounds i and j , and σ_i and σ_j are the standard deviations of the peak for the compounds i and j , respectively.

3. Results and discussion

3.1. NMR spectroscopy

The number of spectral lines, their relative intensity, the position in the spectra and comparison with the known spectra of N-alkyl-2-benzothiazolesulfenamide derivatives unambiguously proved the expected structure of the alkylbisBS compounds presented in Fig. 1. Assignment of the ^{13}C NMR chemical shifts was done by comparison with spectra of N-alkyl-2-benzothiazolesulfenamide derivatives. In the case of possible ambiguity of the results, H,H and H,C correlation experiments were exploited. The starting points in the assignment were the well-defined resonances of the quaternary car-

bons C_1 and C_6 . Based on the long-range 3-bond H,C correlations with these carbon protons, H_3 and H_4 could be unambiguously discriminated. ^1H multiplet resonances are severely overlapping. Using H,H-COSY and one-bond H,C-COSY all other proton and carbon resonances of the aromatic ring were identified. Whereas ^1H resonances of alkylbis(2-benzothiazolylsulfen)amides are severely overlapping, their ^{13}C resonances are clearly distinguishable. Carbon spectra proved to be useful for the reliable and quick identification of alkylbisBS compounds in the mixtures, also in the presence of other benzothiazole derivatives such as N-alkyl-2-benzothiazole-sulfenamides, 2,2'-dithio-bisbenzothiazole and benzothiazole. Data are shown in Table 1.

3.2. RP-HPLC

A reversed-phase HPLC technique was chosen for the simultaneous determination of isopropyl-, *tert.*-butyl-, *tert.*-amyl-, and cyclohexylbis(2-benzothiazolesulfen)amides. Two types of C_{18} bonded-phase columns were used for the separation, i.e. Nucleosil 120-3 C_{18} with 7 μm particle diameter and Separon SGX C_{18} with 7 or 5 μm particle diameter.

Though all alkylbisBS derivatives are very well soluble in diethyl ether and tetrahydrofuran, these solvents are not suitable in our case. In the presence of these solvents, the elution curves of

Table 1
 ^{13}C NMR chemical shifts (δ/ppm) of alkylbis(2-benzothiazolylsulfen)amides

C_i/alkyl	Isopropyl	<i>tert.</i> -Butyl	<i>tert.</i> -Amyl	Cyclohexyl
C-1	154.1	154.0	153.8	154.2
C-2	121.2	121.2	121.2	121.1
C-3	124.4	124.4	124.5	124.3
C-4	126.5	126.2	126.2	126.2
C-5	122.3	122.3	122.4	122.2
C-6	135.4	135.5	135.6	135.3
C-7	172.6	173.2	173.0	173.0
C-8	67.3(C-q)	69.3(C-q)	72.3(C-q)	74.3(CH)
C-9	22.5(CH_3)	29.5(CH_3)	34.0(CH_2)	32.9(CH_2 ,o)
C-10	–	–	29.8(CH_3 -1)	25.6(CH_2 ,m)
C-11	–	–	26.4(CH_3 -2)	25.0(CH_2 ,p)

all derivatives on the columns described above and eluted with acetonitrile–methanol–water or acetonitrile–water were asymmetric and peak widths were doubled. The same phenomenon was found when tetrahydrofuran was only a part of the mobile phase. NMR analysis showed that in diethyl ether and tetrahydrofuran no isomers or other compounds occurred. Finally, acetone proved to be a suitable solvent for our analysis.

The mobile phase composition used for the simultaneous determination of all derivatives of alkylbisBS and the calculated capacity factors are summarized in Tables 2 and 3. Retention times and capacity factors were measured at a flow-rate of $0.8 \text{ cm}^3 \text{ min}^{-1}$. All alkylbisBS derivatives had two absorption maxima, at ca. 230 and 280 nm.

On the chosen columns elution with methanol–water did not result in the separation of the alkylbisBS derivatives.

On the Nucleosil 120 $3C_{18}$ ($7 \mu\text{m}$) and Separon SGX C_{18} ($7 \mu\text{m}$) columns eluted with acetonitrile–water (7–8:3–2, v/v) we were able to separate all alkylbisBS derivatives, but resolution of isopropylbisBS from *tert.*-butylbisBS ($R_s = 0.9$) was not sufficient for quantitative analysis. Resolution of these two derivatives was improved by using a ternary mobile phase, acetonitrile–methanol–water (5:3.5:1.5, v/v).

Table 2

Capacity factors of alkylbis(2-benzothiazolylsulfen)amides at various compositions of the mobile phase at a flow-rate of $0.8 \text{ cm}^3 \text{ min}^{-1}$ and λ_{max} 230 nm on the Separon SGX C_{18} column with particle diameter $7 \mu\text{m}$

Mobile phases									
Water	30	30	20	30	15	21	21	15	15 ^a
MeOH	70	0	0	0	35	49	49	35	35 ^a
MeCN	0	70	80	0	0	0	30	50	50 ^a
THF	0	0	0	70	50	30	0	0	0
AlkylbisBS									
IPbisBS	NA	6.6	5.5	– ^b	– ^b	– ^b	4.1	5.5	4.0
TBbisBS	NA	7.3	6.2	– ^b	0.6	2.0	4.6	6.3	4.3
TAbisBS	NA	9.6	8.3	– ^b	1.0	– ^b	6.0	8.1	5.7
CHbisBS	NA	14.1	13.6	0.4	– ^b	3.6	8.6	11.6	9.1
diCHBS	– ^b	17.9	– ^b	– ^b	– ^b	– ^b	11.0	– ^b	10.5

NA: not achieved.

^a Nucleosil 120- $3C_{18}$, $7 \mu\text{m}$ column.

^b Not present in the samples.

Table 3

Capacity factors of alkylbis(2-benzothiazolylsulfen)amides at various composition of the mobile phase at a flow-rate of $0.8 \text{ cm}^3 \text{ min}^{-1}$ and λ_{max} 233 nm on the Separon SGX C_{18} column with particle diameter $5 \mu\text{m}$

Mobile phases							
Water	30	20	9	13	15	18	21
MeOH	0	0	21	17	15	12	9
MeCN	70	80	70	70	70	70	70
AlkylbisBS							
IPbisBS	19.3	13.1	3.9	6.0	7.1	9.9	10.7
TBbisBS	22.6	15.2	4.3	6.5	8.1	11.3	12.4
TAbisBS	30.3	19.4	5.4	9.1	10.4	14.8	16.3
CHbisBS	45.0	25.8	7.5	11.7	14.9	21.7	23.8
diCHBS	55.8	34.9	9.1	15.1	18.3	26.7	29.4

However, as shown in Table 4, the resolution for this problematic couple of derivatives was only 1.2, still not sufficient enough for quantitative determination. Under the conditions used the resolution for this couple of alkylbisBS derivatives could not be improved by changing the flow-rate of the mobile phase, as shown in Table 4.

Substantially better results were achieved on a Separon SGX C_{18} $5 \mu\text{m}$ column. As shown in Table 3, using acetonitrile–water (7–8:2–3, v/v) as the mobile phase, the capacity factors were

Table 4

Resolution of alkylbis(2-benzothiazolylsulfen)amides at various flow-rates of the mobile phase MeCN–MeOH–H₂O (50:35:15, v/v) at λ_{\max} 230 nm on the Separon SGX C₁₈ column with particle diameter 7 μm

	Flow-rate (cm ³ min ⁻¹)				
	0.6	0.7	0.8	0.9	1.0
alkylbisBS					
IPbisBS	1.1	1.1	1.2	1.0	0.8
TBbisBS	2.8	2.8	2.9	2.5	1.8
TABisBS	4.9	5.1	5.4	4.2	3.1
CHbisBS					

high. The capacity factor can be lowered by gradient elution of acetonitrile and water or, as shown below, by a ternary mobile phase of acetonitrile–methanol–water under isocratic condition. Because of the smaller and simpler equipment needed, we chose the isocratic procedure for the determination. A suitable combination of acetonitrile–water with methanol allows to separate all alkylbisBS derivatives quickly and effectively with excellent resolution of isopropylbisBS from *tert.*-butylbisBS, as the problematic couple of derivatives. We found (see Table 3) that replacing part of the water (up to 15 vol.%) in the acetonitrile–water (7:3, v/v) mobile phase by methanol, decreased the capacity factors of the alkylbisBS derivatives. However, the resolution for the couple of IPbisBS and TBbisBS derivatives was not decreased below the limit value $R_s = 1.5$. By changing the

Table 5

Resolution of alkylbis(2-benzothiazolylsulfen)amides at various flow-rates of the mobile phase MeCN–MeOH–H₂O (70:15:15, v/v) at λ_{\max} 233 nm on the Separon SGX C₁₈ column with particle diameter 5 μm

	Flow-rate (cm ³ min ⁻¹)				
	0.8	1.0	1.2	1.4	1.6
alkylbisBS					
IPbisBS	1.9	1.7	1.6	1.5	1.4
TBbisBS	4.5	4.0	3.9	3.7	3.5
TABisBS	7.1	6.8	6.0	6.0	6.0
CHbisBS	4.3	4.2	3.7	3.7	3.7
diCHBS					

flow-rate of the mobile phase acetonitrile–methanol–water (7:1.5:1.5, v/v), as shown in Table 5, we were also able to shorten the elution times of the alkylbisBS derivatives. The time of analysis is similar or shorter compared with that of the gradient method.

N-Dicyclohexyl-2-benzothiazolesulfenamide proved to be a suitable internal standard for our analysis. In Fig. 2 a chromatogram of all alkylbisBS derivatives is shown with the internal standard using acetonitrile–methanol–water (7:1.5:1.5, v/v) as the mobile phase at the flow-rate of 1.4 cm³ min⁻¹ and detection at λ_{\max} 233 nm.

The detection limit using acetonitrile–methanol–water at a flow-rate of 1.4 cm³ min⁻¹ and detection at $\lambda_{\max} = 233$ nm, is $1.1 \cdot 10^{-4}$ AU. The determined limits of quantitation for IPbisBS, TBbisBS, TABisBS, CHbisBS and diCHBS are 127, 118, 128, 151 and 166 ng cm⁻³, respectively.

The precision of the internal standard method for all alkylbisBS derivatives was determined with freshly prepared samples using acetonitrile–methanol–water (7:1.5:1.5, v/v) as the mobile phase at a flow-rate of 1.4 cm³ min⁻¹ and detection at $\lambda_{\max} = 233$ nm. Samples were in contact with acetone no longer than 4 h at ambient temperature. NMR analysis confirmed that after this time a slow decomposition of these compounds occurred. The precision was estimated from the analysis of a sample containing

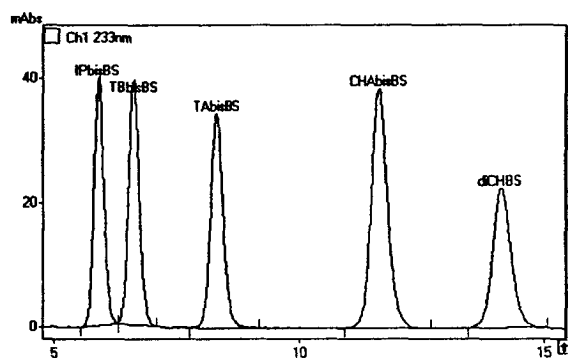


Fig. 2. Chromatogram of alkylbis(2-benzothiazolylsulfen)amides with N-dicyclohexyl-2-benzothiazolesulfenamide as internal standard using acetonitrile–methanol–water (7:1.5:1.5, v/v) as mobile phase at the flow-rate of 1.4 cm³ min⁻¹ and λ_{\max} 233 nm.

Table 6

Determined amounts of separated alkylbis(2-benzothiazolylsulfen)amides on the Separon SGX C₁₈ column with particle diameter 5 μm eluted with mobile phase AcCN–MeOH–H₂O (7:1.5:1.5, v/v) at a flow-rate of 1.4 cm³ min⁻¹ and λ_{max} 233 nm

<i>n</i>	Component				
	IPbisBS (wt.%)	TBbisBS (wt.%)	TAbisBS (wt.%)	CHbisBS (wt.%)	Grade (wt.%)
1	22.81	21.05	23.21	32.56	99.63
2	22.20	21.40	23.59	32.82	100.01
3	22.58	21.08	23.51	32.43	99.60
4	22.03	21.25	23.50	32.39	99.17
5	22.72	20.99	23.64	32.74	100.09
6	23.05	21.20	23.71	32.48	100.44
7	22.52	21.16	23.39	32.77	99.84
Mean (wt.%)	22.55	21.16	23.50	32.59	99.82
S.D.	0.35	0.14	0.16	0.17	–
R.S.D. (%)	1.55	0.66	0.68	0.52	–
Real value (μg cm ⁻³)	11.7	11.0	12.2	16.9	51.8
Real value (wt.%)	22.59	21.24	23.55	32.62	100.00

about 12 μg cm⁻³, i.e. approximately 25 wt.% of each alkylbisBS derivative. The determined areas were recalculated to the content (wt.%) of the component in the samples by the software used. The R.S.D. of 7 replicate injections for all alkylbisBS derivatives was within 1.5% (Table 6).

The method presented here has been found to be suitable for the analysis of alkylbisBS derivatives in waste water and other solvents used in their manufacture. The analyses are not interfered with raw material or by-products, such as N-alkyl-2-benzothiazolesulfenamides, 2,2'-dithiobisbenzothiazole and benzothiazole, that may be present in samples.

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