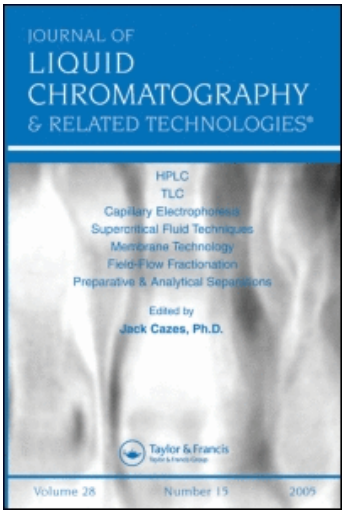

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF 5-DODECYLSALICYLALDOXIME AND 3-CHLORO-5-DODECYLSALICYLALDOXIME, TWO LIPOPHILIC ANALOGUES OF SALICYLALDOXIME

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ABSTRACT

High-performance liquid chromatographic analyses are reported for 5-dodecylsalicylaldoxime (5-DO-SAO) and 3-chloro-5-dodecylsalicylaldoxime (3-Cl-5-DO-SAO), two lipophilic analogues of salicylaldoxime. A reversed-phase column (Supelcosil LC-18, 150 x 4.6 mm, 5 μm mean particle size) was used as the stationary phase and a mixture of tetrahydrofurane and water as the mobile phase (volume ratio 70:30 for 5-DO-SAO and 72:28 for 3-Cl-5-DO-SAO). UV absorption at 226 nm was used for detection. The retention times of the analytes were 6.0 and 5.8 min, respectively, with a flow rate of 0.5 ml/min. For both compounds, the limit of detection was 0.5 μg/ml. The methods were linear up to 400 and 500 μg/ml, respectively.

INTRODUCTION

Trans-bis(salicylaldoximato)copper(II) ($CuSAO_2$), i.e. the copper(II) chelate of salicylaldoxime (2-hydroxybenzaldoxime; SAO), is a potent antiproliferative agent that essentially totally prevents tumor cell proliferation *in vitro* in very low concentrations and also has potent, even curative, antitumor activity *in vivo* [1-3]. The very low aqueous solubility of the chelate, however, is a serious disadvantage concerning practical therapeutic applications. In order to obtain more soluble analogues, the corresponding copper(II) chelates of certain di- and trihydroxybenzaldoximes were synthesized [1,2] but even these compounds were found to be almost insoluble in water. The immunomodulating properties [4] and, especially, the organ selectivity of this class of antineoplastic compounds (a nearly specific affinity for the pancreas, with almost 1000-fold accumulation in this organ as compared to the liver, kidneys and heart) [5] have further increased the interest in the agents. Therefore, studies are in progress in our laboratory in order to find out novel water-soluble or fat-soluble, yet antineoplastic, analogues of the potentially highly valuable but difficult-to-use agents. In this connection, fat-soluble analogues of SAO are of great potential

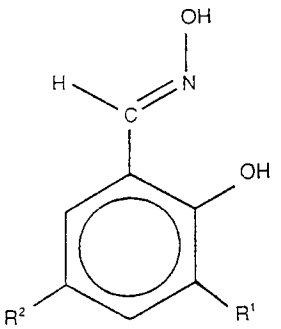

FIGURE 1. The structures of SAO ($R^1 = R^2 = H$), 5-DO-SAO ($R^1 = H$, $R^2 = n$-dodecyl) and 3-Cl-5-DO-SAO ($R^1 = Cl$, $R^2 = n$-dodecyl).

interest. In the present paper, we report high-performance liquid chromatographic analyses for 5-dodecylsalicylaldoxime (5-DO-SAO) and 3-chloro-5-dodecylsalicylaldoxime (3-Cl-5-DO-SAO), two fat-soluble analogues of SAO bearing long aliphatic side chains (see Fig. 1 for structures of SAO and the analogues now studied). The present compounds are commercially used in the mining industry. If they are to be used as starting materials in the synthesis of analogues of $CuSAO_2$ for antitumor screening purposes or for potential therapeutic applications, analytical methods are needed for them.

EXPERIMENTAL

Chemicals

Samples of 5-DO-SAO and 3-Cl-5-DO-SAO were kindly donated by Henkel Corporation, Tucson, AZ, U.S.A. HPLC grade tetrahydrofurane (THF) was obtained from Rathburn (Walkerburn, Scotland). Water was purified by using a Millipore Alpha Q water purification system.

Chromatographic Apparatus and Conditions

All chromatographic measurements were carried out using a high-performance liquid chromatographic system consisting of two LC-10AD liquid chromatograph solvent delivery systems and an SPD-M6A photodiode-array UV-VIS detector (Shimadzu Corporation, Kyoto, Japan). A Hyundai Super 386N Plus computer equipped with a 120 MB hard disk and 4 MB extended memory was used for data acquisition and processing in this system, employing Shimadzu LC workstation [Class-LC10 version 1]. The disk operating system was MS-DOS version 5.0. Injection was done using a Shimadzu SIL-6B auto injector, controlled by a SCL-6B system controller.

A Supelcosil LC-18 column (catalog no. 5-8230, 150 x 4.6 mm I.D., 5μm mean particle size), obtained from Supelco, Inc., Supelco Park, Bellefonte, PA, U.S.A., was employed. A LiChroCART 4-4 (Cat. 50957) precolumn containing LiChrospher 100 RP-18 packing material (particle size 5 μm) was used and was obtained from E. Merck (Darmstadt, Germany).

Chromatographic separations were carried out at room temperature using isocratic systems with a THF-water mixture as the mobile phase, the volume ratio of the solvents being 70:30 in the case of 5-DO-SAO and 72:28 in the case of 3-Cl-5-DO-SAO. The components of the eluents were degassed with the aid of helium. The injection volume was 20 μl, the analytes being dissolved in a mixture of water and THF (volume ratio 1:1). The analytes can also be dissolved in 100 % THF but in that case, a prominent extra peak appears in the chromatograms. A constant flow rate of 0.5 ml/min was used. Detection was based on UV absorption at 226 nm, measured with the diode array detector.

RESULTS AND DISCUSSION

Just as in the case of the HPLC analysis of SAO and 2,4-dihydroxybenzaldoxime (β-resorcylaldoxime) [6], mixtures of THF and water were found to be suitable as the mobile phase also for the lipophilic SAO analogues now studied, although a clearly higher concentration of THF was found to be necessary in the case of the latter compounds. When THF-water mixtures were employed as the eluent, the best results for samples of commercial 5-DO-SAO were obtained with a THF/water volume ratio 70:30, while in the case of the 3-chloro-5-dodecyl congener, a slightly higher THF content gave optimal results (volume ratio 72:28). Typical chromatograms obtained using these conditions are shown in Figs. 2 and 3, respectively. In the chromatogram of 5-DO-SAO, an intense peak was observed at 6.0 min and a very small one at 5.0 min. In the chromatogram of 3-Cl-5-DO-SAO, an intense peak was analogously

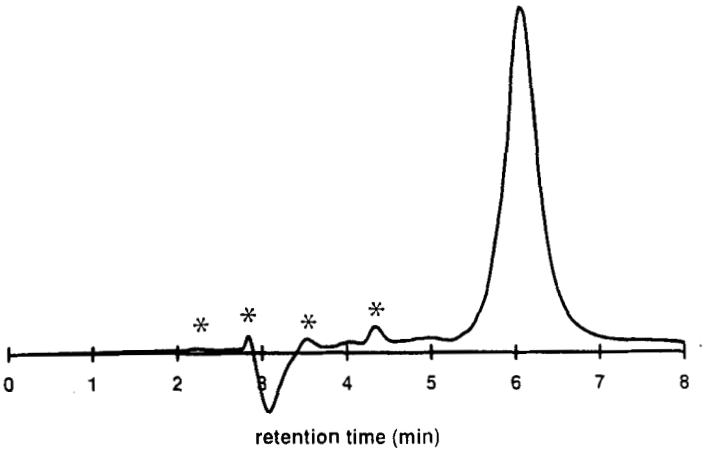

FIGURE 2. Chromatogram of a sample of commercial 5-DO-SAO dissolved in a 1:1 mixture of water and THF (99 μg of 5-DO-SAO/ml; for conditions, see Experimental). * = peaks that appear also in the chromatogram of the mere solvent (water plus THF 1:1).

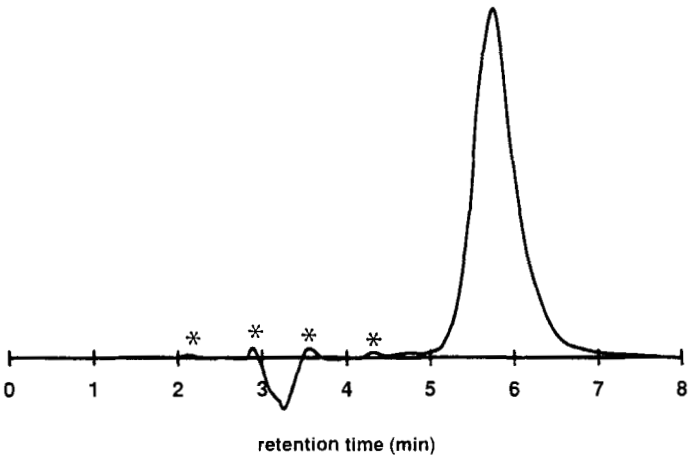

FIGURE 3. Chromatogram of a sample of commercial 3-Cl-5-DO-SAO dissolved in a 1:1 mixture of water and THF (98 μg of 3-Cl-5-DO-SAO/ml; for conditions, see Experimental). * = peaks that appear also in the chromatogram of the mere solvent (water plus THF 1:1).

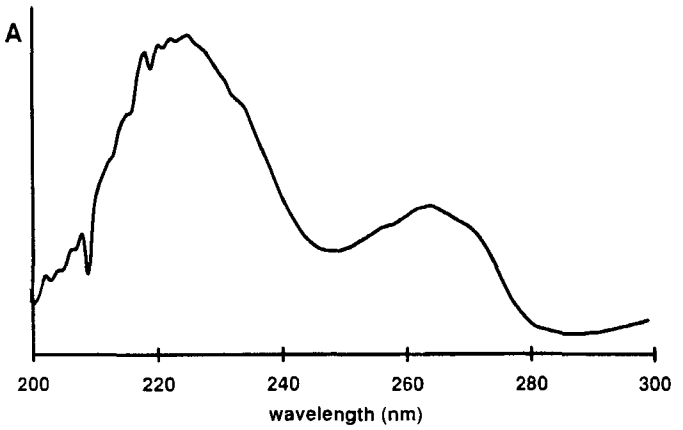

FIGURE 4. UV spectrum of 5-DO-SAO (recorded with the aid of the diode array detector at the top of the peak appearing at 6.0 min in the chromatogram of a sample whose concentration was 95 μg/ml).

observed at 5.8 min and a very small one at 4.7 min. With higher THF concentrations, the above mentioned small peaks that were observed under the conditions described above were no more separated from the intense peaks. When lower THF concentrations were used, the analyte peaks were either broadened or no peaks could be observed.

In the case of both analytes, UV spectra recorded at the intense peaks were largely similar to the spectrum of pure authentic SAO (see Figs. 4-6). In the case of the two compounds studied, spectra that were recorded at the above mentioned small peaks were largely similar to each other but totally different from the spectrum of SAO and from the spectra recorded at the intense peaks. Thus, the intense peaks at 6.0 and 5.8 min could be identified as the analyte peaks. Peak purity indexes obtained from spectra recorded at the peak top, the up slope and the down slope of the intense peaks indicated that, in the case of both compounds, the analyte peak is obviously due to one compound only. The small peaks observed at 5.0 and 4.7 min are obviously due to impurities, whose identity

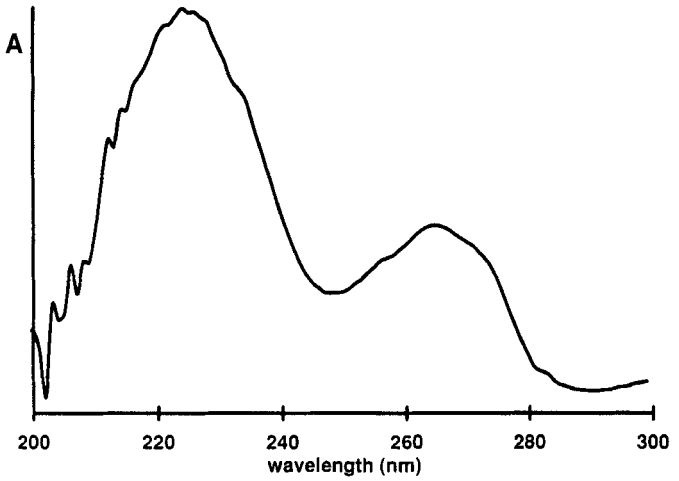

FIGURE 5. UV spectrum of 3-Cl-5-DO-SAO (recorded with the aid of the diode array detector at the top of the peak appearing at 5.8 min in the chromatogram of a sample whose concentration was 98 μg/ml).

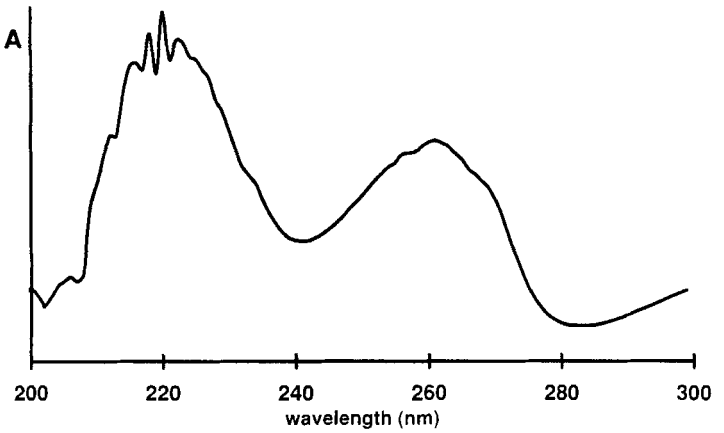

FIGURE 6. UV spectrum of SAO. The spectrum was recorded with the aid of the diode array detector at the top of the SAO peak of a chromatogram of pure authentic SAO. The chromatographic apparatus and conditions were those described in [6].

TABLE 1

Intra-Day Repeatabilities of the Methods for Standard Samples at Three Concentrations

Compound	n	Concentration (μg/ml)	Coefficient of Variation (% of Mean Peak Area)	Coefficient of Variation (% of Mean Peak Height)
5-DO-SAO	6	10	3.7	4.2
3-Cl-5-DO-SAO	6	11	1.2	2.1
5-DO-SAO	6	99	1.4	2.1
3-Cl-5-DO-SAO	6	98	2.4	1.8
5-DO-SAO	6	198	1.8	1.3
3-Cl-5-DO-SAO	6	194	2.0	2.0

TABLE 2

Inter-Day Repeatabilities of the Methods for Standard Samples at Three Concentrations

Compound	n	Concentration (μg/ml)	Coefficient of Variation (% of Mean Peak Area)	Coefficient of Variation (% of Mean Peak Height)
5-DO-SAO	15	10	5.3	5.8
3-Cl-5-DO-SAO	15	11	2.9	3.2
5-DO-SAO	15	99	3.5	3.0
3-Cl-5-DO-SAO	15	98	1.8	2.0
5-DO-SAO	15	198	3.8	3.3
3-Cl-5-DO-SAO	15	194	2.5	2.5

remains to be studied. Also the low UV absorption intensity of the small peaks, as compared to SAO and 2,4-dihydroxybenzaldoxime [6], indicates that these peaks are not due to the SAO analogues studied.

In the chromatogram of 5-DO-SAO, one further very small peak could be observed at 4.0. min. The UV spectrum of this peak was largely similar to that of the obvious analyte peak, but its very low intensity indicates that the peak must be due to an impurity only. The spectrum suggests that this impurity is probably a structural analogue (possibly an isomer) of 5-DO-SAO, perhaps 3-dodecyl-SAO that would be expected to be formed in the synthesis of the analyte.

For both analytes, the limit of detection was 0.5 µg/ml. The methods were linear up to 400 µg/ml for 5-DO-SAO (r= 0.9998 as based on peak areas at a total of 5 different concentrations, and 0.999994 as based on peak heights) and 500 µg/ml for 3-Cl-5-DO-SAO (r= 0.9997 and 0.9996, respectively, as based on 6 different concentrations). The intra-day and inter-day repeatabilities of the methods developed are good (see Tables 1 and 2).

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