Journal of Chromatography, 147 (1978) 496–500

CHROM. 10,266

Note

High-performance liquid chromatographic determination of halogen-substituted salicylanilides

P. CUKOR, C. PERSIANI, M. WOEHLER and N. BISCHAK
GTE Laboratories, Inc., 40 Sylvan Road, Waltham, Mass. 02154 (U.S.A.)
(Received June 1st, 1977)

Halogen-substituted salicylanilides have been used as long lasting germicidal additives in soaps and disinfectants[1,2]. Photoallergic reactions have been reported in connection with the use of such soaps[3–5]. Certain individuals will develop skin rashes when exposed to UV light after using soaps containing halogen substituted salicylanilides. These compounds were recently removed from the market because of this harmful side effect. It has been postulated that one or more halogen atoms is cleaved via a free radical mechanism from the parent compound upon UV radiation. This photo-induced free radical will combine with proteins, and the new molecule in turn can act as an antigen *in vivo*[6–8].

There is a definite need for chromatographic analysis of various halogen-substituted salicylanilides. Thin-layer chromatography and ion-exchange chromatography have been used to determine the purity of these salicylanilide compounds[9–11]. Chromatography should also prove to be a valuable tool in the investigation of the photochemical reactions of these compounds. Halogenated salicylanilides are high molecular weight, low volatility compounds and as such they are best separated by high-performance liquid chromatography (HPLC). This paper describes an HPLC technique for the determination of brominated salicylanilide derivatives.

EXPERIMENTAL

Materials and apparatus

Separations were carried out with a Waters Assoc. Model 202 liquid chromatograph capable of operating at pressures up to 6000 p.s.i. The instrument incorporated a dual detection system that measured both refractive index and UV absorbance changes. Since the brominated salicylanilides are strong UV absorbers, measurements were made with the UV detector set at 254 nm.

Reversed-phase separations were performed on a Partisil 10-ODS microparticulate column, 4.6 mm × 25 cm, fitted with a 2-μm stainless-steel frit. This C_{18} silane type of bonded packing material was found to be stable through a pH range of 4–12. Prepacked columns are available through Whatman (Clifton, N.J., U.S.A.).

Sample of 20 μl were introduced into the chromatograph via the U6K universal loop injector, using a 50-μl syringe. All samples were filtered through a 0.45-μm pore size HAWP01300 Millipore filter. A flow-rate of 1 ml/min was used and this produced a column pressure of 1000 p.s.i.

The mobile phase was a methanol–water (7:3) mixture which was employed under isocratic conditions. The methanol was glass distilled and purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). 3,4′,5-Tribromosalicylanilide (TBS), 4′,5-dibromosalicylanilide (DBS) and salicylanilide were obtained from Fine Organics (Lodi, N.J., U.S.A.), 4′-monobromosalicylanilide (MBS) was received from Aldrich (Milwaukee, Wisc., U.S.A.).

Procedures

Irradiations. UV irradiations were performed with a UVL-56 black-ray long-wavelength lamp manufactured by Ultra-Violet Products (San Gabriel, Calif., U.S.A.). Samples in a petri dish were exposed to UV light while being stirred at a distance of 5 cm from the light source at an intensity of 2.54 mW/cm² for 2 h. Light intensity was measured by a UVA meter manufactured by GTE Sylvania (Danvers, Mass., U.S.A.).

Methanol–water medium. Halogenated salicylanilide stock solutions were prepared by dissolving the appropriate compound in methanol to give a concentration of 0.5 mg/ml. Aliquots were evaporated and diluted with methanol–water (7:3) to establish a calibration curve in the range of 0.01–0.1 mmole/ml.

Irradiation experiments were conducted on samples of TBS dissolved in the methanol–water (7:3) mixture. The TBS concentration was 0.03 mg/ml.

Ethanol–buffer medium. Calibration curves for TBS, DBS, MBS and salicylanilide in ethanol–buffer system were generated by adding aliquots of the stock solutions of the appropriate compounds in ethanol to 5 ml of a buffer solution. The buffer was made up by combining a solution of 0.1 *M* $Na_2HPO_4 \cdot NaH_2PO_4$ with 0.15 *M* NaCl and adjusted to pH 7.5.

Samples of TBS in ethanol–buffer were also irradiated in a petri dish while being stirred. After irradiation, the solutions were evaporated to dryness and redissolved in acetone. This procedure reduces the amount of buffer salts in solution and was repeated several times to ensure minimum salt concentration. After the last evaporation was completed, the residue was redissolved in the methanol–water (7:3) mixture and was prepared for HPLC analysis.

Ethanol–buffer–protein medium. Human serum albumin stock solution was prepared by dissolving the protein in an ethanol–buffer solution to give a concentration of 5 mg/ml. Known amounts of salicylanilide compounds were added to 5 ml of the protein solution. Acetone was used to precipitate the protein and to reduce the salt concentration. Calibration curves were constructed. Irradiation of TBS and protein in buffer solution was performed as before. Using acetone precipitation, the protein-bound TBS was removed from solution and analysis was performed on the supernatant liquid containing TBS and its light degradation products which were not attached to protein molecules. The supernatant liquid was evaporated and the residue redissolved in methanol–water solution for HPLC analysis.

RESULTS AND DISCUSSION

A typical chromatogram of bromine-substituted salicylanilides is shown in Fig. 1. Calibration curves for each of the materials are presented in Fig. 2. The limit of detection for all materials was 0.005 mg/ml.

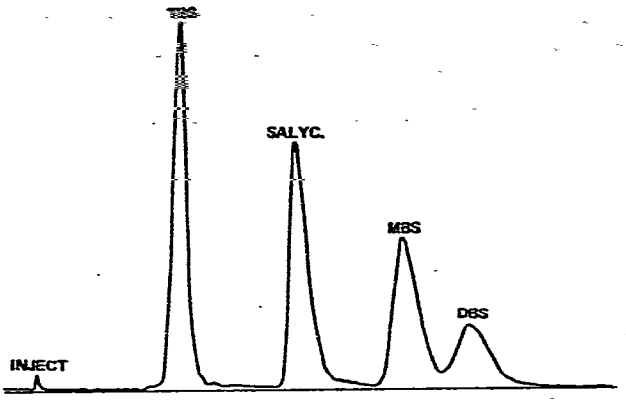

Fig. 1. Typical liquid chromatographic separation of brominated salicylanilides and salicylanilide (SALYC).

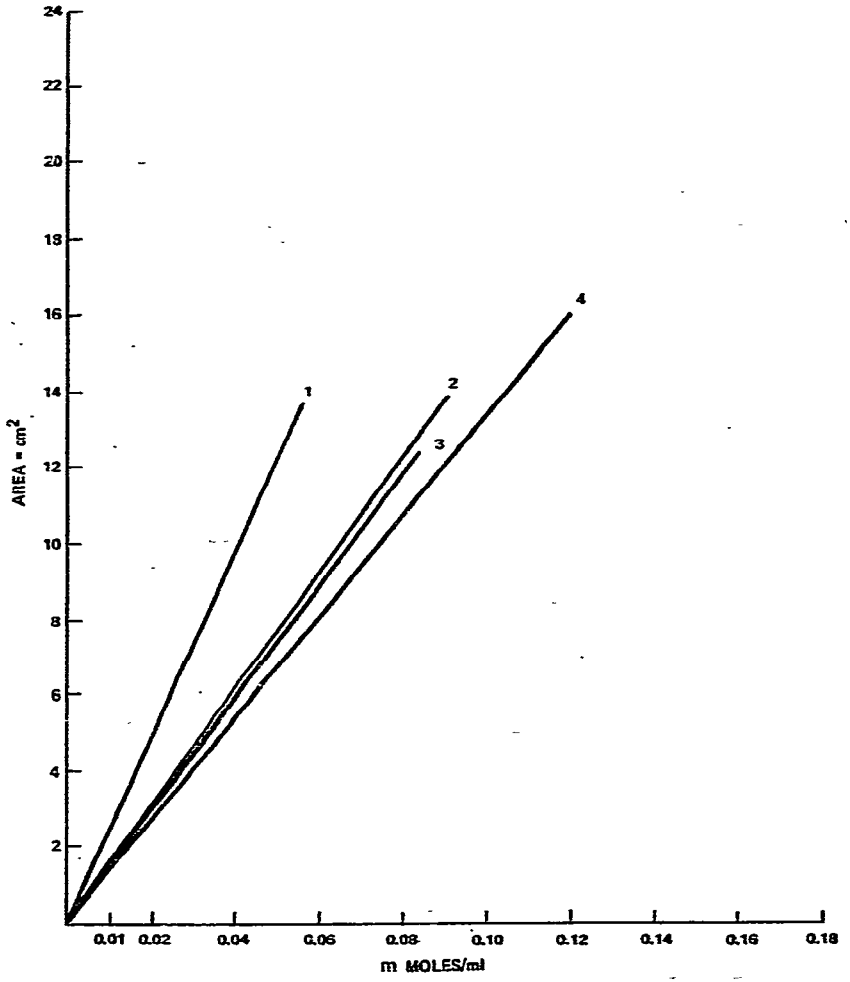

Fig. 2. Calibration curves for TBS(1), MBS(2), DBS(3) and salicylanilide(4).

The method has been used to assess the purity of halogenated salicylanilides. An interesting instance of this was the analysis of commercial DBS which contained 40% TBS (Fig. 3).

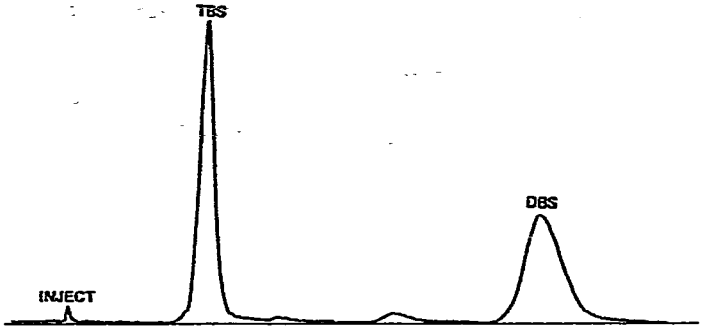

Fig. 3. Liquid chromatogram of commercial dibromosalicylanilide.

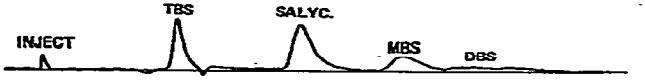

Fig. 4. Photolysis products of TBS.

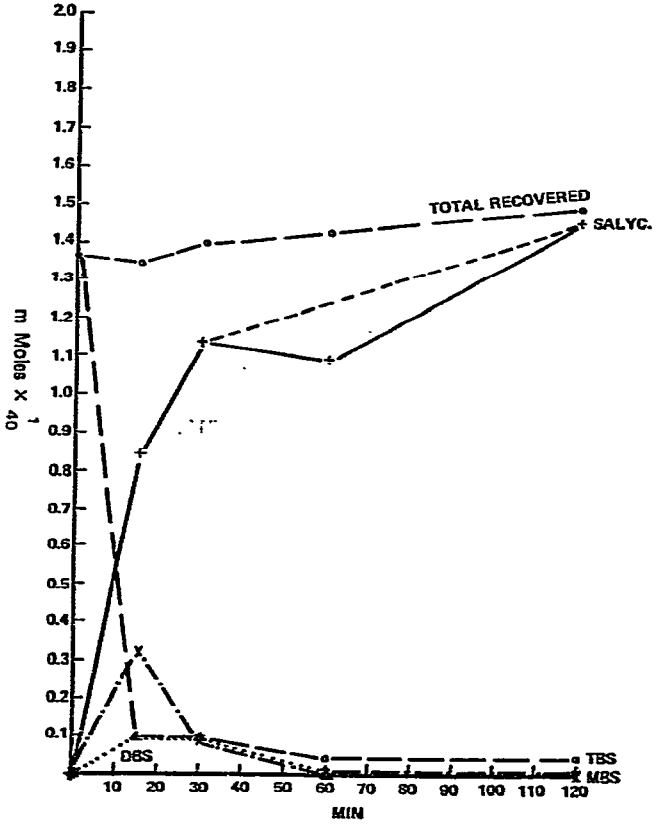

Fig. 5. Product distribution of TBS in buffer–alcohol solution *vs.* irradiation time.

The photodegradation of halogenated salicylanilides was studied in great detail by Welti[12] and Coxon, Jenkins and Welti[13]. They have found that 3,5,4'-tribromosalicylanilide in alcohol solution upon irradiation with 360-nm light yielded 5,4'DBS. The same compound, however, in an alcohol solution of higher pH produced upon irradiation 4'-MBS. We have repeated these experiments and found that TBS in alcohol solution indeed yielded DBS only. We observed, however, that in alcohol–buffer solution of pH 7.5, all possible degradation products, *i.e.*, DBS, MBS and salicylanilide are formed. Fig. 4 shows the chromatograms obtained after photolysis and Fig. 5 indicates the product distribution as a function of irradiation time in alcohol–buffer solution.

CONCLUSIONS

A liquid chromatographic method has been developed for the separation and determination of brominated salicylanilides. The limit of detection was 0.005 mg/ml. Bromine-substituted salicylanilides have been used in soaps as bacteriocides and have been found to cause photoallergy. The chromatographic method reported in this paper can be used to facilitate the assay of halogenated salicylanilides by determining their presence in soaps and is also useful in studying the mechanism of the photoallergic reaction.

REFERENCES

1 Hankel and Cie, *German Pat.*, 1,568,910 (1969).
2 Unilever, N.V., *Dutch Pat.*, 6,914,605 (1969).
3 P. E. Osmundsen, *Boll. Chim. Farm.*, 110 (1971) 590.
4 M. D. Alani, *Brit. J. Dermatol.*, 89 (1973) 361.
5 D. S. Wilkinson, *Brit. J. Dermatol.*, 73 (1961) 213.
6 F. P. Jenkins, D. Welti and D. Baines, *Nature (London)*, 201 (1964) 827.
7 W. Burckhardt, *Dermatologica*, 83 (1941) 63.
8 L. C. Hurber and R. L. Baer, *J. Invest. Dermatol.*, 58 (1972) 327.
9 N. E. Skelly and K. A. Kanke, *Anal. Chem.*, 39 (1967) 1010.
10 N. E. Skelly and W. B. Crummett, *Anal. Chem.*, 35 (1963) 1680.
11 F. W. Baker and G. E. Booth, *Proc. Joint Conf. Cosmet. Sci.*, (1968) 57.
12 D. Welti, *Spectrochim. Acta*, 19 (1963) 655.
13 J. A. Coxon, F. P. Jenkins and D. Welti, *Photochem. Photobiol.*, 4 (1965) 713.