Journal of Chromatography, 596 (1992) 127–131
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 988

Short Communication

Studies on photoisomerization of 4,4′-diaminostilbene-2,2′-disulphonic acid for quality assurance by high-performance liquid chromatography☆

Sajid Husain*, R. Narsimha, S. N. Alvi and R. Nageswara Rao

Analytical Chemistry Division, Indian Institute of Chemical Technology, Hyderabad-7 (India)

(First received November 18th, 1991; revised manuscript received December 31st, 1991)

ABSTRACT

Studies on the photoisomerization of 4,4′-diaminostilbene-2,2′-disulphonic acid (DAST) were carried out using controlled light at 350 nm to follow the *E* to *Z* isomerization and to develop a simple and rapid stability-indicating assay using high-performance liquid chromatography. Photoisomers and process impurities, *viz.*, 4,4′-diaminodibenzyl-2,2′-disulphonic acid and 4,4′-dinitrostilbene-2,2′-disulphonic acid, were separated using a reversed-phase C_{18} column, an eluent containing aqueous ammonium sulphate and 2-propanol and a variable-wavelength UV spectrophotometric detector at 245 and 338 nm. The method was used for quality assurance and validated using several lots of industrial samples. The mean recovery of DAST from authentic samples was 99.75 ± 1.17%.

INTRODUCTION

The *E*-isomer of 4,4′-diaminostilbene-2,2′-disulphonic acid (DAST) is an important intermediate in the manufacture of dyes, optical brighteners and fluorescent whitening agents [1]. It is manufactured [2,3] generally by the reduction of 4,4′-dinitrostilbene-2,2′-disulphonic acid (DNST) using iron and hydrochloric acid. During this process, small amounts of 4,4′ diaminodibenzyl-2,2′-disulphonic acid (DADB) are obtained as a by-product [4]. Owing to the similarities in solubility characteristics and chemical properties, it is difficult to separate DADB from DAST. This is considered to be the most objectionable impurity if the material is to be used for manufacturing optical whitening agents. Further, aqueous solutions of DAST undergo rapid isomerization [5] on exposure to light or heat, yielding the *Z*-isomer. Therefore, techniques for the determination of its purity are important in determining not only the yield but also the performance of the final product.

Few methods for the quality assurance of DAST have been reported. Titrimetry and potentiometry [6,7] have been extensively used, but these methods are neither specific nor selective for DAST. Interferences from impurities and isomerization of DAST have made spectrophotometric methods [8–10] unreliable. Ion-exchange chromatography [11] has been applied but failed to separate *E*- and *Z*-isomers. Thin-layer chromatography [12] has been applied to the determination of DAST in river water. Studies on the occupational exposure of fabric brightening agents and the determination of DAST in air by ion-pair high-performance liquid chromatography [13] have been performed. However, it is

☆ IICT Communication No. 2932.

0021-9673/92/$05.00

known [14] that the use of ion-pair reagents such as tetrabutylammonium dihydrogenphosphate and cetyltrimethylammonium bromide in analyses for aromatic sulphonic acids often results in peak splitting and irreproducible peak shapes. However, this could be overcome [15–18] using solutions of inorganic salts as mobile phases.

In this investigation, studies on the photoisomerization of DAST were performed. A simple and rapid HPLC method for the quality assurance of DAST using a μBondapak C_{18} column and an eluent containing 0.15 *M* aqueous ammonium sulphate and 2-propanol at ambient temperature was developed.

EXPERIMENTAL

Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. HPLC-grade 2-propanol was obtained from Spectrochem (Bombay, India) and ammonium sulphate from BDH (Poole, UK).

DAST was prepared by heating 4-nitrotoluene-2-sulphonic acid in sodium hydroxide solution until a deep red colour was obtained and then it was reduced with iron and hydrochloric acid [2]. Technical-grade samples of DAST were obtained from Vasant Chemicals (Hyderabad, India).

Studies on the photoisomerization of DAST were carried out by exposing aqueous solutions to UV light at 350 nm using a Raynot photochemical reactor.

Apparatus

A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a 20-μl loop injector and a high-pressure six-way valve was used. A Shimadzu SPD-6AV variable-wavelength UV–VIS spectrophotometric detector was connected after the column. A μBondapak C_{18} (Waters Assoc., Milford, MA, USA) column (300 mm × 3.9 mm I.D.; particle size 10 μm) was used for separation. The chromatograms and the integrated data were recorded with a Chromatopac C-R3A processing system.

Chromatographic conditions

The mobile phase was 0.15 *M* aqueous ammonium sulphate–2-propanol (98:2, v/v). Samples were dissolved in small volumes of 0.02% aqueous sodium hydroxide and diluted with sufficient volumes of the mobile phase. Analyses were carried out under isocratic conditions at a flow-rate of 1 ml/min and a chart speed of 5 mm/min at room temperature (27°C). Chromatograms were recorded at 245 nm for 5 min and then at 338 nm using a wavelength-programmable UV detector.

Analytical procedure

Samples of DAST (10 mg) were dissolved in the mobile phase (25 ml) and a 20-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures and commercial formulations were analysed under identical conditions. The percentage of DAST was calculated from the peak area.

RESULTS AND DISCUSSION

The HPLC separation of DAST and its impurities is shown in Fig. 1. The peaks were identified by injecting the individual compounds. It can be seen that DAST is resolved not only from the process impurities but also from its *Z*-isomer. 2-Propanol was used as an organic solvent modifier to improve

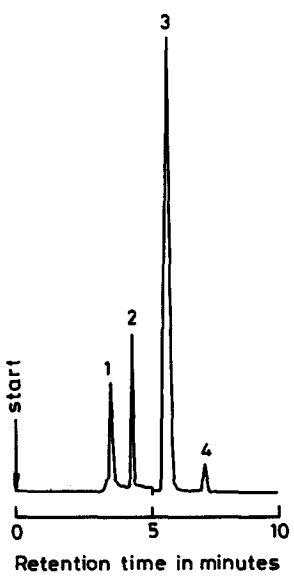

Fig. 1. HPLC profile of a typical mixture containing (1) (*Z*)-DAST (2.0 μg), (2) DADB (2.0 μg), (3) (*E*)-DAST (5.0 μg) and (4) DNST (1.0 μg). For conditions, see text.

TABLE I

RETENTION DATA

Compound	Retention time (min)	Relative retention time	λ_{max} (nm)
(*Z*)-DAST	3.67	0.52	248
DADB	4.63	0.66	245
(*E*)-DAST	5.68	0.81	338
DNST	7.03	1.00	330

TABLE II

DETECTOR RESPONSE FOR DAST AND DADB

Compound	Amount (10^{-7} g)	Area	Relative standard deviation (%)	n^a
DAST	1.57	16 272	1.89	5
DADB	1.02	11 045	2.54	5

[a] Number of measurements.

the separation. Earlier attempts using acetonitrile resulted in overlapping of the peaks of DAST and DADB.

The wavelengths of maximum absorption and retention times, for all the compounds are given in Table I. Two different wavelengths, *i.e.*, 245 nm for 5 min and then 338 nm, were used for detection not only because the detection of each component is ensured but also because good linearity between mass and integral response is obtained. The response data for DAST and DADB are given in Table II. When the UV detector is set at 0.001 a.u.f.s.

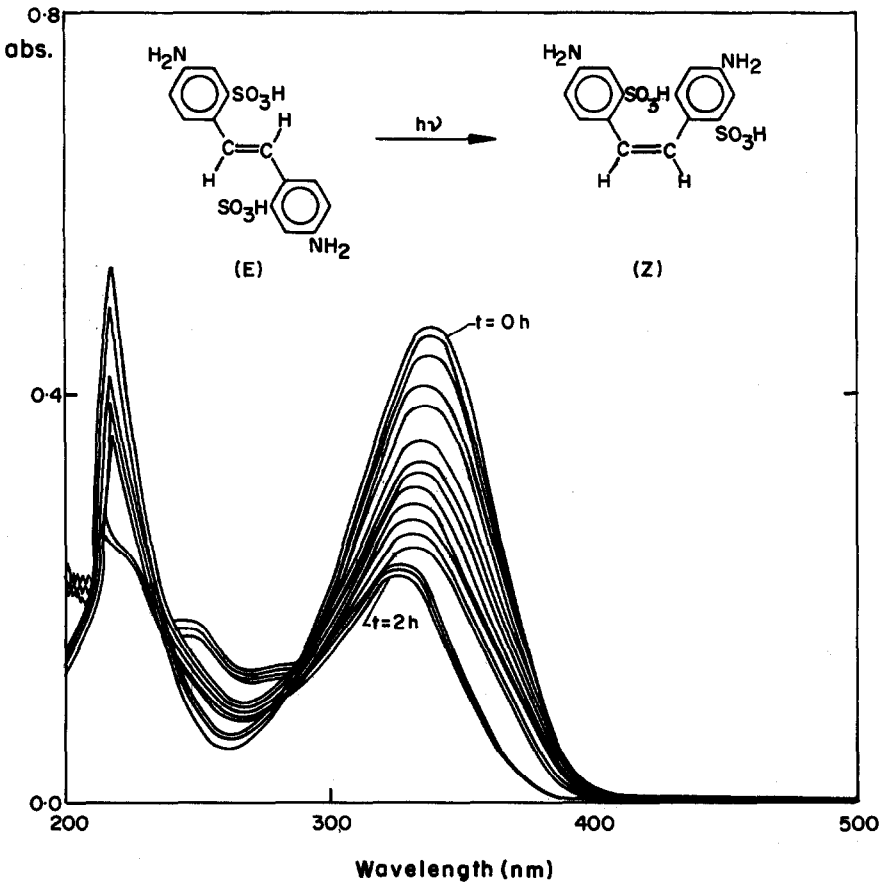

Fig. 2. Changes in the UV absorption spectrum of a $1.4 \cdot 10^{-5}$ *M* solution of DAST in NaOH on exposure to daylight.

TABLE III
UV AND ^{1}H NMR SPECTRAL DATA FOR (*E*)- AND (*Z*)-DAST

DAST	Conformation	λ_{max} (nm)	Chemical shift ppm
Before exposure to light	*E*	338	6.91, 6.93, 7.00, 7.03, 7.66, 7.71, 7.76
After exposure to light	*Z*	245	6.43, 6.46, 6.52, 6.55 6.82, 6.92, 7.03, 7.20, 7.32

TABLE IV
STABILITY-INDICATING ASSAY OF DAST

Irradiation time (h)	(*E*)-DAST (10^{-3} g)	(*Z*)-DAST (10^{-3} g)	Conversion (%)
0.00	5.18	0.00	0.00
0.25	4.37	0.84	15.63
0.50	2.14	3.04	58.68
1.00	1.90	3.28	63.32
2.00	1.83	3.35	64.67

the limit of detection for DAST is 5.0×10^{-9} g with a signal-to-noise ratio of 4.0.

Fig. 2 shows the consecutive changes in the UV absorption spectrum of DAST undergoing photochemical rearrangement. The λ_{max} was gradually shifted from 338 nm to 245 nm. The intensity of absorption also decreased significantly. These changes have been attributed to *E* to *Z* isomerization of DAST using ^{1}H NMR spectrometry. The λ_{max} and ^{1}H NMR chemical shift data of DAST before and after irradiation are given in Table III. The conversion was followed quantitatively by HPLC. The effect of light on the stability of DAST can be seen clearly from the chromatograms shown in Fig. 3. The conversion data are given in Table IV. The half-life of (*E*)-DAST is *ca.* 0.5 h on exposure to sunlight. These results indicate that aqueous solutions of DAST are highly sensitive to light, which affects the assay of DAST significantly.

Standards containing different amounts of DAST were prepared and analysed by HPLC. The measured amounts agreed well with the actual values (within 1.36%). The mean recovery of DAST was 99.75 ± 1.17%. Linear regression analysis of the data yielded the line $y = 0.9839x + 0.0658$ with a correlation coefficient of 0.9986 (y = amount of DAST found; x = amount of DAST taken). Technical and commercial preparations of DAST (Fig. 4) were analysed by the proposed method and the results, given in Table V, are in good agreement with those claimed by the manufacturer (within 1.43%).

It is concluded that the proposed method is suitable not only for the quality assurance of DAST but also to establish the proportion of each isomer resulting from photoisomerization.

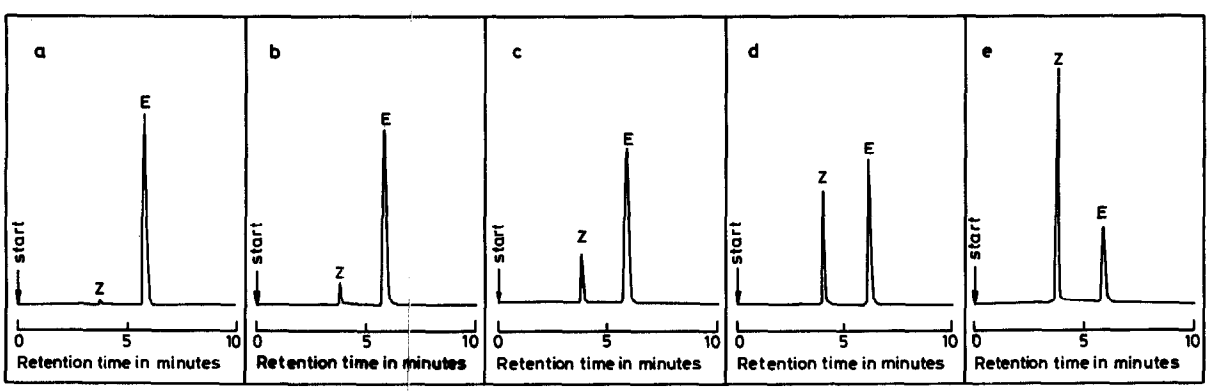

Fig. 3. Chromatograms showing the effect of light on the stability of (*E*)-DAST: (a) before irradiation; (b), (c), (d) and (e) after 0.25, 0.50, 1.00 and 2.00 h of irradation, respectively.

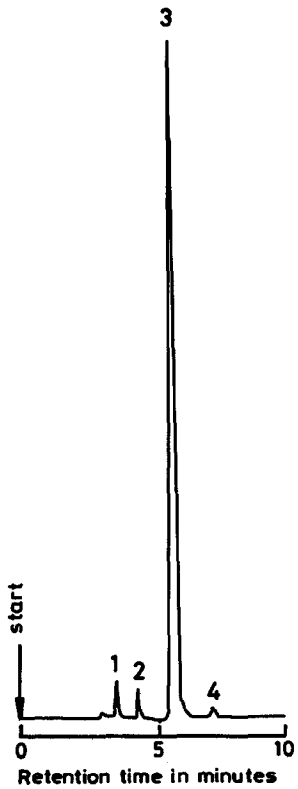

Fig. 4. Chromatogram of a commercial sample of DAST. For identification of peaks, see Fig. 1.

TABLE V

DETERMINATION OF DAST IN TECHNICAL AND COMMERCIAL PREPARATIONS

Code	Mean DAST content (%) ± R.S.D.[a] (%)		Error (%)
	Present[b]	Found[c]	
SD1	65.08 ± 2.17	65.93 ± 1.53	+ 1.31
SD2	98.62 ± 1.50	97.85 ± 1.96	− 0.78
VC1	72.57 ± 1.94	73.61 ± 1.82	+ 1.43
VC2	97.94 ± 1.08	98.58 ± 0.97	+ 0.65

[a] Relative standard deviation ($n = 5$).
[b] By UV spectrometry.
[c] By HPLC.

REFERENCES

1 S. Budavari (Editor), *The Merck Index*, Merck, Rahway, NJ, 11th ed., 1989, p. 95.
2 K. Venkatraman, in L. F. Fieser and M. Fieser (Editors), *The Chemistry of Synthetic Dyes*, Vol. 1, Academic Press, New York, 1952, pp. 628–629.
3 S. Bender, *Chem. Ber.*, 19 (1886) 3234.
4 R. E. Farris, in *Kirk-Othmer, Encyclopedia of Chemical Technology*, Vol. 21, Wiley-Interscience, New York, 1983, pp. 729–746.
5 Y. Yoshio, *Yuki Gosei Kagaku Kyokai Shi*, 30 (1972) 818.
6 *Specifications for 4,4'-Diaminostilbene-2,2'-disulphonic Acid*, IS: 4265, Indian Standards Institution, New Delhi, 1972, pp. 6–7.
7 H. Norwitz and P. N. Keliher, *Talanta*, 35 (1986) 311.
8 B. Stzeke, *Organika*, (1986) 31; *C.A.*, 101 (1984) 203641.
9 J. Barek and I. Danhel, *Collect. Czech. Chem. Commun*, 49 (1984) 2751.
10 E. J. Woodhouse, E. A. Murril, K. M. Stelting, R. D. Brown and C. W. Jameson, in C. W. Jameson and D. B. Walters (Editors), *Problems of Testing Commercial Grade Chemicals*, Butterworth, Boston, 1984, pp. 31–49.
11 D. J. Subach, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, (1979) 633.
12 A. Abe and H. Yoshimi, *Water Res.*, 13 (1979) 1111.
13 S. K. Hammond, T. U. Smith and M. J. Ellenbeckee, *Am. Ind. Hyg. Assoc. J.*, 48 (1987) 117.
14 H. U. Ehmeke, H. Kelker, K. H. Konigs and H. Ullner, *Fresenius' Z. Anal. Chem.*, 294 (1979) 251.
15 P. Jandera and H. Engelhardt, *Chromatographia*, 13 (1980) 18.
16 P. Jandera, J. Churacek and B. Taraba, *J. Chromatogr.*, 262 (1983) 121.
17 A. Zein and M. Baerms, *J. Chromatogr. Sci.*, 27 (1989) 249.
18 C. D. Gaitonde and M. U. Pathak, *J. Chromatogr.*, 514 (1990) 330.