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Monitoring the products of acetylation, sulphonation and condensation of 2,4-diaminobenzenesulphonic acid by high-performance liquid chromatography☆

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ABSTRACT

A simple and rapid high-performance liquid chromatographic method was developed for the separation and determination of 2,4-diaminobenzenesulphonic acid (DASA) in acetylation, sulphonation and condensation products. The separation was achieved on a reversed-phased μBondapak C_{18} column using 0.15 *M* ammonium sulphate–acetonitrile (80:20, v/v) as the eluent. The method was used not only for quality assurance but also for process development of 2,4-DASA and was validated using several industrial samples. The mean recovery of DASA from authentic samples was 99.86 ± 1.54% and the limit of detection was $5 \cdot 10^{-9}$ g.

INTRODUCTION

2,4-Diaminobenzenesulphonic acid (DASA) is an important intermediate in the manufacture of dyes for cotton, wool, leather and cosmetics [1]. It is produced in large amounts by the sulphonation of 2,4-dinitrochlorobenzene (DNCB) followed by reduction using iron and hydrochloric acid [2,3]. It yields valuable products such as 2-amino-4-acetanilidobenzenesulphonic acid (AASA), 2,4-diaminobenzenedisulphonic acid (DADA) and 4-aminobenzamide-N-(3-aminobenzene-4-sulphonic acid) (ABSA) on acetylation, sulphonation and condensation respectively [4–6]. Unreacted DASA is generally present in small amounts as an impurity of these compounds, reducing the quality of the finished products significantly. Owing to the similarities in solubility characteristics and chemical properties it is difficult to separate and determine DASA. The separation and determination of DASA are therefore important not only for quality assurance of these products but also for their process development.

A literature search revealed that no method has been reported for the quality assurance of DASA, AASA, DADA and ABSA. Chemical methods of analysis based on diazotization and coupling suffer from interferences from impurities and isomerization. Paper and thin-layer chromatographic methods have been widely used, but are qualitative in nature [7,8]. High-performance liquid chromatography is the method of choice for the separation of aromatic sulphonic acids [9,10]. Cellulose- and polystyrene-based ion-exchange columns have been used extensively to study the behaviour of aromatic sulphonic acids [11], but the separations on these columns have been found to be unsatisfactory because of the poor recovery of analytes due to strong adsorptions and hydrophobic inter-

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actions. Zou *et al.* [12] were successful in overcoming these difficulties using ion-pair reagents in reversed-phase liquid chromatography. However, the use of ion-pair reagents such as tetrabutylammonium hydrogensulphate and cetyltrimethylammonium bromide should generally be avoided not only because of the added complexity of the mobile phase but also because of baseline artifacts, irregular peak shapes and widths, marked sensitivity of separations to temperature and slow equilibration of the column before and after ion-pair chromatography [13]. Jandera *et al.* [14,15] reported reproducible and more economical separations using reversed-phase columns with aqueous inorganic salts as mobile phases. In this investigation we extended this idea to the determination of the title compounds not only for quality assurance but also for process development.

In this paper, we describe a simple and rapid high-performance liquid chromatographic method for the separation and determination of small amounts of DASA in reaction mixtures and final products of DADA, AASA and ABSA using a μBondapak C_{18} column and an eluant containing 0.15 *M* ammonium sulphate at ambient temperature.

EXPERIMENTAL

Materials and reagents

All reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled water, HPLC-grade acetonitrile (Spectrochem, Bombay, India) and ammonium sulphate (BDH, Poole, UK) were used. DASA was prepared by heating 2,4-dinitrochlorobenzene with sodium sulphite and then reducing it with iron and hydrochloric acid. AASA, DADA and ABSA were prepared by acetylation, sulphonation and condensation of DASA with acetic anhydride, sulphuric acid and 4-nitrobenzoyl chloride, respectively. Technical-grade samples of DASA, DADA, AASA and ABSA were obtained from Orchem Intermediates (Hyderabad, India).

Apparatus

A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) with a 20-μl loop injector having a six-way high-pressure 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 × 3.5 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* ammonium sulphate–acetonitrile (80:20, v/v). Samples were dissolved in the mobile phase. The analysis was 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 the corresponding absorption maxima (λ_{max}) of the eluting compounds using a wavelength-programmable UV detector.

Analytical procedure

Samples (10 mg) were dissolved in the mobile phase (100 ml) and a 20-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures and technical and commercial formulations were analysed under identical conditions. The percentage of DASA was calculated from the peak area.

RESULTS AND DISCUSSION

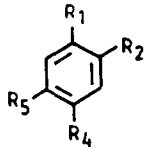
The structures of the compounds under investigation are given in Table I. Fig. 1 shows the reaction pathways followed for preparation of DASA, DADA, AASA and ABSA.

Quality assurance of DASA

The HPLC separation of DASA and its impurities is shown in Fig. 2. The peaks were identified by injecting the individual compounds. It can be seen that DASA is well resolved from the process reactants, *i.e.*, 2,4-DNCB and DNSA. Impurities, *i.e.*, 2,6-DNCB and 2,6-DASA do not interfere in the determination as they elute at 14.50 and 7.12 min, respectively. Acetonitrile was used as an organic solvent modifier to improve the separation. Earlier attempts using different columns, *i.e.*, μBondapak

TABLE I

CHEMICAL STRUCTURES AND ABBREVIATIONS OF THE COMPOUNDS UNDER INVESTIGATION



Compound	Abbreviation	R_1	R_2	R_4	R_5
2,4-Dinitrochlorobenzene	DNCB	Cl	NO_2	NO_2	H
Sodium 2,4-dinitrobenzenesulphonate	DNSA	SO_3Na	NO_2	NO_2	H
2,4-Diaminobenzenesulphonic acid	DASA	SO_3H	NH_2	NH_2	H
2,4-Diaminobenzene-1,5-disulphonic acid	DADA	SO_3H	NH_2	NH_2	SO_3H
2-Amino-4-acetanilidobenzenesulphonic acid	AASA	SO_3H	NH_2	$NHCOCH_3$	H
2,4-Diacetanilidobenzenesulphonic acid	DABA	SO_3H	$NHCOCH_3$	$NHCOCH_3$	H
4-Aminobenzamide-N-(3-aminobenzenesulphonic acid)	ABSA	SO_3H	NH_2	$NHCOC_6H_4NH_2$	H

C_8 and μBondapak CN, with aqueous triethylamine and acetonitrile resulted in overlapping of the peaks of DNSA and DNCB.

The effect of temperature on the separation was studied at 20, 25 and 30°C. It was found that a change in the temperature of the column of ±5°C has no significant effect on either the retention capacity or the resolution obtained between DASA and DNSA. However, the elution time of 2,4-DNCB was found to be reduced by 1.5 min per 5°C raise in temperature, resulting in a low resolution ($\alpha_{20°C} = 3.72$, $\alpha_{25°C} = 3.25$ and $\alpha_{30°C} = 2.69$) between DNSA and 2,4 DNCB.

The wavelengths of maximum absorption and retention times for DASA, DNSA and DNCB are given in Table II. Three different wavelengths, *i.e.*, 220 nm for 5 min, 252 nm for 10 min and then 245 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 these compounds are included in Table II. When the UV detector is

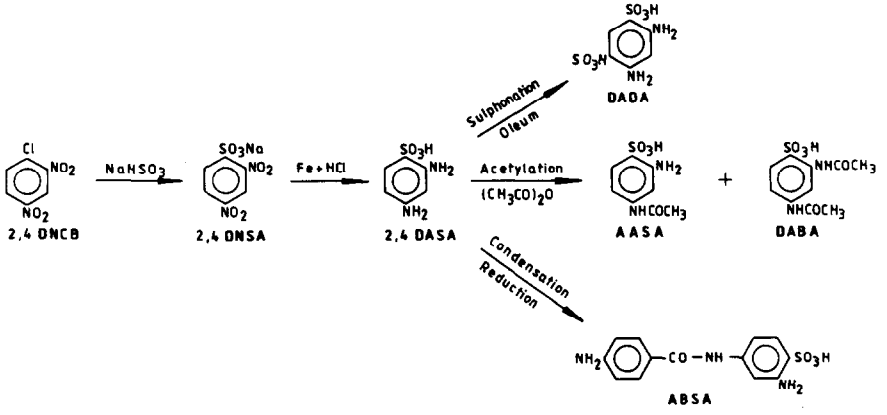


Fig. 1. Preparation of 2,4-diaminobenzenesulphonic acid (DASA) and its products of sulphonation, acetylation and condensation.

TABLE II

RETENTION TIMES (t_R), RELATIVE RESPONSE FACTORS (*RF*), WAVELENGTHS OF MAXIMUM ABSORPTION (λ_{max}) AND LINEARITY DATA FOR DASA, DNSA AND DNCB

Compound	t_R (min)	*RF*	λ_{max} (nm)	Linearity data	
				Regression equation[a]	Correlation coefficient
DASA	4.88	1.29	220	$y = 0.976x + 0.035$	0.998
DNSA	6.07	1.00	252	$y = 0.998x - 0.012$	0.995
DNCB	12.43	2.33	245	$y = 0.979x + 0.023$	0.997

[a] x = Amount taken; y = amount found.

set at 0.001 AUFS the limit of detection for DASA is $5.0 \cdot 10^{-9}$ g with a signal-to-noise ratio of 4.0.

Standards containing known amounts of DASA, DNSA and DNCB were prepared and analysed by HPLC. The accuracy of the method was determined by the standard addition technique. Subsequent additions of small amounts of the impurities were accurately reflected in their peak heights. The measured amounts agreed well with the actual values within 1.45%. The response factors for 1 μg each of all the compounds were determined and used to establish the composition of samples obtained during process development.

The method was applied to monitor the process conditions. The yield of DNSA in the first step was studied. It was separated by adding salts slowly to the mother liquor. The recovery was found to be around 72%. The filtrate containing unrecovered DNSA, unreacted DNCB and salts is called the "effluent" and it is generally disposed of to the environment after treatment in industry. These effluents were collected and analysed by HPLC. It was found that DNSA is present in the effluent in significant amounts and it has to be recovered economically from the effluent. This may be accomplished either by solid-phase extraction or by adjusting the pH. DNSA is further reduced to DASA using iron and hydrochloric acid. It may contain 2-nitro-4-aminobenzenesulphonic acid as an impurity.

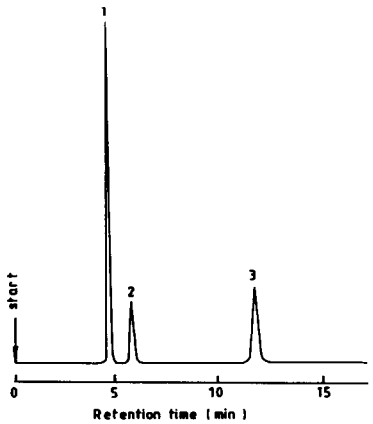


Fig. 2. Chromatogram of a typical mixture containing (1) DASA (20 μg), (2) DNSA (10 μg) and (3) DNCB (10 μg).

The quality of DASA was thoroughly checked on several lots of samples received from industry. The concentrations of various impurities were determined by HPLC and the purity of DASA was calculated. The results are given in Table III and show that the method is precise and accurate. Similar calculations were also

TABLE III

ANALYTICAL DATA FOR TYPICAL SAMPLES OF DASA RECEIVED FROM INDUSTRY

Sample	Assay (%)	S.D.[a] (%)
OIPL/1/92	0.972	1.26
OIPL/2/92	0.986	1.52
OIPL/1/93	0.989	1.07
OIPL/2/93	0.995	1.43

[a] $n = 3$.

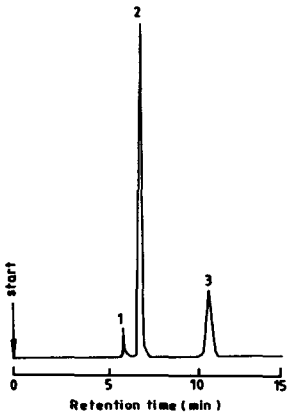


Fig. 3. Chromatogram of a typical mixture containing (1) DASA (2 μg), (2) AASA (20 μg) and (3) DABA (5 μg).

carried out for quality assessment of acetylation, sulphonation and condensation products of DASA. The precision of the purity values thus obtained was found to be good (within 1.63%) in all instances.

Acetylation of DASA

DASA was reacted with acetic anhydride to yield AASA (Fig. 1). Depending on the conditions of the reaction, it generally contains DABA as a by-product and DASA as an unreacted impurity. HPLC separation of these compounds is shown in Fig. 3. The retention data are presented in Table IV. The levels of DASA in reaction mixtures and the final product were determined by HPLC. The results are recorded in Table V. It can be seen that DASA levels as low as 0.1% can be determined accurately by HPLC.

TABLE IV

RETENTION TIMES (t_R), DETECTOR RESPONSES AND WAVELENGTHS OF ABSORPTION MAXIMA (λ_{max}) OF DASA, AASA AND DABA

Compound	t_R (min)	Relative response factor	λ_{max} (nm)
DASA	5.75	1.00	220
AASA	6.68	1.13	228
DABA	10.27	1.96	238

Sulphonation of DASA

DASA was treated with oleum to obtain DADA. The reaction mixture was collected and analysed by HPLC. The HPLC profile is shown in Fig. 4. The peaks were identified by injecting individual authentic compounds. DASA and DADA were found to elute at 5.41 and 8.43 min, respectively. Other reagents, *viz.*, chlorosulphonic acid and sulphuryl chloride, were also tried for sulphonation of DASA and the products obtained were analysed by HPLC. The results are given in Table V.

Condensation of DASA

Initially DASA was condensed with *p*-nitrobenzoyl chloride and then reduced to ABSA using iron and hydrochloric acid. All the reactants of this process were subjected to HPLC and their separation is shown in Fig. 5. It can be clearly seen that DASA is well separated not only from ABSA but also from other impurities, *viz.*, PNBC and NBSA. The method has been well standardized and used for process development. It has been found to be helpful not only for monitoring the reactions but also for improving significantly the yield and purity of ABSA (Table V).

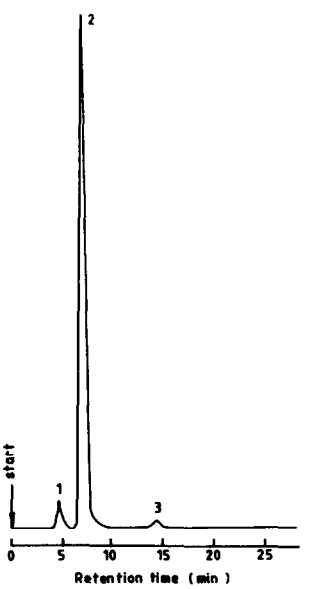


Fig. 4. Chromatogram of the product obtained on sulphonation of DASA. Peaks: 1 = DASA; 2 = DADA; 3 = unknown.

TABLE V

LEVELS OF DASA DETERMINED IN SAMPLES OF AASA, DADA AND ABSA BY HPLC

Sample	DASA concentration (%)	R.S.D.[a] (%)
AASA, Expt. 1	0.34	2.45
AASA, Expt. 2	1.93	2.08
AASA, Expt. 3	3.47	1.59
AASA, Expt. 4	5.45	1.23
DADA, Expt. 1	0.37	2.73
DADA, Expt. 2	0.72	2.15
DADA, Expt. 3	1.83	1.98
ABSA, Expt. 1	0.78	2.80
ABSA, Expt. 2	1.58	1.97

[a] $n = 3$.

CONCLUSIONS

A simple and rapid HPLC method employing a reversed-phase C_{18} column has been developed for monitoring the acetylation, sulphonation and condensation products of DASA. It is precise and accurate for the separation and determination of small amounts of DASA in several of the dye intermediates, *viz.*, AASA, DADA and ABSA. The method is suitable not only for process development but also for quality assurance of DASA and related products.

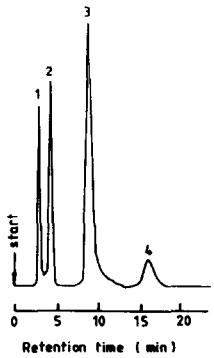


Fig. 5. Chromatogram of the reaction mixture collected during the course of condensation followed by reduction of DASA. Peaks: 1 = DASA; 2 = ABSA; 3 = PNBC; 4 = NBSA.

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