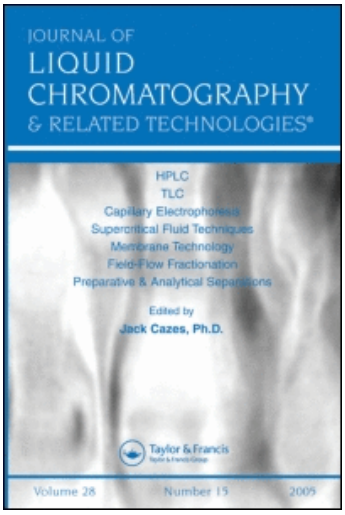
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Sodium Benzoxazole-2-Sulfonate: A Derivatisation Reagent for the Analysis of Amines and Amino Acids by HPLC With Fluorescence or UV Detection

O. R. Idowu[a]; G. O. Adewuyi[b]

[a] Department of Pharmacology Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. [b] Department of Chemistry, University of Ibadan, Ibadan, Nigeria

To cite this Article Idowu, O. R. and Adewuyi, G. O.(1993) 'Sodium Benzoxazole-2-Sulfonate: A Derivatisation Reagent for the Analysis of Amines and Amino Acids by HPLC With Fluorescence or UV Detection', Journal of Liquid Chromatography & Related Technologies, 16: 17, 3773 — 3791

To link to this Article: DOI: 10.1080/10826079308019666

URL: http://dx.doi.org/10.1080/10826079308019666

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(17), 3773-3791 (1993)

SODIUM BENZOXAZOLE-2-SULFONATE: A DERIVATISATION REAGENT FOR THE ANALYSIS OF AMINES AND AMINO ACIDS BY HPLC WITH FLUORESCENCE OR UV DETECTION*

O. R. IDOWU**[1] AND G. O. ADEWUYI[2]
[1]*Department of Pharmacology*
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100
[2]*Department of Chemistry*
University of Ibadan
Ibadan, Nigeria

Downloaded At: 08:13 25 January 2011

ABSTRACT

The preparation of sodium benzoxazole-2-sulfonate and its use as a reagent for the formation of fluorescent derivatives of amines and amino acids is described. Sodium benzoxazole-2-sulfonate is readily prepared by boiling 2-chlorobenzoxazole with sodium sulfite. Sodium benzoxazole-2-sulfonate is non-fluorescent, but reacts with amines and amino acids to give derivatives which exhibit intense blue

*This article originally appeared in Volume 16, Number 12, 1993, pages 2501-2518. Due to a printing error which resulted in figures being transposed between two articles, it is being reprinted here in its entirety.

**To whom all correspondence should be addressed.

Copyright © 1993 by Marcel Dekker, Inc.

fluorescence. The solubility of the compound in water also makes it particularly advantageous as a reagent for the derivatisation of amino acids. Furthermore, sodium benzoxazole-2-sulfonate may be tagged onto an ion-exchange resin, in which form it may be used for heterogeneous on-line pre- or post-column derivatisation of amines and amino acids.

INTRODUCTION

A substantial proportion of analytical methods based on HPLC rely on the derivatisation of the analyte for improved detectability with the ultraviolet (UV) and fluorescence detectors which are the most widely used detectors for HPLC. Derivatisation has been of particular importance in the HPLC analysis of aliphatic amines and amino acids because these compounds have very low UV detection sensitivities and they absorb in the inaccessible, short-wavelength region where other compounds, equally lacking in chromophoric groups, also absorb. Fluorescence detection of these compounds is possible only after their conversion to fluorescent derivatives.

Amines have been converted to UV-absorbing or fluorescent derivatives for the purpose of HPLC analysis by a variety of approaches [1,2]. Fluorescent derivatives are much favoured because they can be detected not only with greater or equal sensitivity as UV-absorbing derivatives, but with greater specificity.

Most chemical derivatisation of amines and amino acids to give fluorescent compunds make use of homogeneous chemical reactions in which a solution of the analyte is mixed with a solution of the required fluorogenic reagent and the mixture is chromatographed after being subjected to suitable reaction conditions of temperature and time. Two of these homogeneous reactions

Downloaded At: 08:13 25 January 2011

have been adapted for on-line post-column derivatisation of amine and amino acids. The most well-known reagents for the homogeneous deivatisation of aliphatic amines and amino acids are dansyl chloride [3-5], fluorescamine [6], NBD-chloride [7,8] and o-phthalaldehyde [9-12]. One of the frustrating disadvantages of homogeneous derivatisation with reagents such as dansyl chloride, which are themselves intensely fluorescent, is due to the close similarity in the spectra or fluorescent characteristics of the reagent and the derivative it forms with amines and amino acids. The inevitable presence of excess reagent in the reaction mixture, therefore, causes high background response and hence, an inadequate detection limit for the analyte. Introduction of steps to remove excess reagent often makes the method more tedious and more prone to error. Non-fluorescent or poorly fluorescent reagents such as fluorescamine, NBD-chloride and o-phthalaldehyde which react with amines to give flourescent derivatives are, in this respect, better than a fluorescent reagent such as dansyl chloride. These reagents, however, share the disadvantage that they can only be used in homogeneous media.

Downloaded At: 08:13 25 January 2011

These and other serious disadvantages of homogeneous derivatisation reactions can be overcome through heterogeneous or solid-phase derivatisation reactions in which the reagent is bound chemically (covalently or ionically) to an inert support such as silica gel or an ion-exchange resin. Krull and co-workers have done substantial work on the development of bound reagents for analytical derivatisation and have discussed the important advantages of solid-phase derivatisation reactions in HPLC [13-15]

Krull and co-workers [16-18] have described covalently bound reagents for the UV or fluorogenic

derivatisation of amines. One of these reagents [16] is a polymeric anhydride containing the o-acetylsalicyl group as the labelling moiety while the others are based on a 9-fluorenylmethyl tag, with the tag being bound to a polymeric backbone through either a benzotriazole linkage[17] or through an ester-carbonate linkage [18]. A common disadvantage of these reagents is their instability towards moisture. Furthermore, these reagents are not readily accessible because their preparation requires the special polymeric supports mentioned above. The reagents have, therefore,not found routine application in HPLC derivatisation of amines or amino acids. Supported reagents in which the reagents are ionically bound to the insoluble polymeric support are to be preferred since readily available ion-exchange resins could be used in their preparation, and they would have a greater shelf-life

The present report is on the use of sodium benzoxazole-2-sulfonate (I, figure 1) as a reagent for the homogeneous derivatisation of amines and amino acids for the purpose of HPLC with fluorescence or UV detection. A solid-phase reagent in which benzoxazole-2-sulfonate is ionically bound to an anion exchange resin can also be prepared for possible use in pre- or post-column derivatisation of amines and amino acids

Downloaded At: 08:13 25 January 2011

MATERIALS AND METHODS

Chemicals: The following chemicals were obtained from British Drug Houses (Poole, England): o-aminophenol, carbon disulfide, phosporus pentachloride, thionyl chloride, sodium sulfite, diethyl-, di-n-propyl-,and -di-n-butylamine, glycine, l-lysine and l-cysteine. Amberlite^{R}-IRA 400 ion-exchange resin (chloride form)

was obtained from Aldrich Chemical Co (Milwaukee, USA). HPLC grade acetonitrile was obtained fromn Burdick and Jackson (Muskagon, MI, USA).

Instrumentation: HPLC was performed on a Waters liquid chromatography system consisting of a Waters model 510 solvent delivery unit, a U6K injector and a Waters model 440 UV detector set at 254 nm. A Waters μBondapak C_{18} column (4.6mm x 30cm) was used together with a mobile phase of acetonitrile:water (65:35 v/v) at a flow rate of 2.0 ml/min. Data acquisition and chromatograph control was done using a Waters 860 data station.

Mass spectrometric identification of the derivatives was performed using a HPLC-MS system consisting of a Hewlett Packard 1090 Liquid Chromatography System linked to a Hewlett Packard HP 5989A Mass Spectrometer through a HP 59980B particle beam interface. The HPLC mobile phase was a 50:50 (v/v) mixture of acetonitrile and 0.1M ammonium acetate(adjusted to pH 4.5) at a flow rate of 0.5 ml/min. There was no column on-line. The conditions of the mass spectrometer was set as follows for the positive EI mode: desolvation temperature, 65°C; source temperature, 150°C; electron energy,230eV; helium pressure, 60 psi; acceleration potential, 7kV. Computerised background subtraction was carried out.

Thin layer chromatography was carried out on silica gel G with acetonitrile:water (85:15 v/v) as mobile phase. The spots were detected by examining the plates under UV light.

Preparation of Sodium benzoxazole-2-sulfonate

Preparation of 2-Mercaptobenzoxazole

A mixture consisting of o-aminophenol (15.0 g), 75 ml of methanol, a solution of 10 g of potassium hydroxide in 20 ml water and 50 ml of carbon disulfide

Downloaded At: 08:13 25 January 2011

was refluxed for 2.5 h after which another 30-50 ml portion of carbon disulfide was added. After refluxing for a further 30 min, the mixture was allowed to cool to room temperature and decolorising charcoal was added. After refluxing for another 10-15 min the mixture was filtered through flutted filter paper. The filtrate was distilled to dryness. The residue was redissolved in 100 ml of water and the solution treated with a solution of 30 ml glacial acetic acid mixed with an equal volume of water. 2-Mercaptobenzoxazole was immediately precipitated as a whitish solid. The solid was filtered and dried in air.

Downloaded At: 08:13 25 January 2011

Preparation of 2-Chlorobenzoxazole and then, Sodium benzoxazole-2-sulfonate

2-Mercaptobenzoxazole (5.0 g) was mixed with about 15 g of phosphorus pentachloride and the mixture heated on a boiling water bath for 1 h and then on a hot plate to reflux for 1.5 h. After cooling to room temperature, the mixture was treated with 50 ml of a 25% (w/v) solution of sodium sulfite. After the vigorous effervescence stopped, a further 50 ml of the sodium sulfite solution was added and the mixture refluxed for 2 h by heating on a hot plate. The mixture was then filtered hot through flutted filter paper. Needle-shaped crystals of sodium benzoxazole-2-sulfonate precipitated as the solution cooled .

Thionyl chloride may be used in place of phosphorus pentachloride. When 5.0 g of 2-mercaptobenzoxazole was treated with 20 ml of thionyl chloride, a vigorous reaction occurred immediately and a dark-green solution was obtained. The solution was refluxed for 2 h, during which three 10 ml portions of thionyl chloride were added at intervals. After distilling off the excess thionyl chloride, a brown oil was obtained. The oil was

mixed with 50 ml of 25% (w/v) solution of sodium sulfite and the mixture refluxed for 2.5 h. The mixture was filtered hot as above. The brown solid left in the flask was repeatedly digested (four times) with 15 ml of water by heating on a hot plate for 10-15 min. On each occassion, the hot solution was filtered into the original filtrate. White needles of sodium benzoxazole-2-sulfonate appeared as the filtrate cooled down.

Preparation of Resin-bound Benzoxazole-2-sulfonate

Air-dried anion-exchage resin (1.0 g) in the chloride form (capacity,2.97 meq/g) was stirred with a solution of 0.696 g of sodium benzoxazole-2-sulfonate in 50 ml of hot water (75°C) for 15 min, after which a 10.00 ml aliquot of the supernatant was taken and titrated with a freshly standardised 0.1M solution of silver nitrate. The procedure was repeated six times but with different mixing times of 30, 60, 90, 120, 150 and 180 min respectively. Comparison of the titre values showed that the amount of sodium chloride released into solution as a result of the displacement of chloride ions by the benzoxazole-2-sulfonate ion remained constant after 30 min of stirring the resin with sodium benzoxazole-2-sulfonate. This was taken to mean that the exchange was complete within 30 min, and subsequent preparations of the resin-bound benzoxazole-2-sulfonate simply involved stirring a warm solution of the reagent with the anion-exchange resin for 30-45 min. The tagged resin was then washed several times with warm water by decantation and then allowed to dry in air.

Reaction of Amines with Sodium benzoxazole-2-sulfonate

Diethlyamine (100 µl) was added to a solution of 0.1 g of sodiun benzoxazole-2-sulfonate in 5.0 ml of water. The mixture was examined under UV light (254 and 360 nm) before being warmed on a water bath at 60°C for

Downloaded At: 08:13 25 January 2011

5 min. After cooling, the mixture was extracted with 5 ml of chloroform by shaking on a vortex mixer for 1-2 min. The aqueous layer was removed with a Pasteur pipet and the chloroform dried with anhydrous sodium sulfate. The chloroform extract was then trasferred to another test tube and the chloroform removed under a stream of nitrogen. Some of the residue obtained was dissolved in methanol for TLC, HPLC and mass spectrometric analysis.The methanol solution was also examined under UV light. The procedure was repeated using di-n-propyl- or di-n-butylamine in place of diethylamine. Dimethylamine hydrochloride (1.0 g) was also reacted with 0.1 g of sodium benzoxazole-2-sulfonate as above, but with the reaction mixture being basified with 1.0 ml of 5M sodium hydroxide solution to release the amine.

Downloaded At: 08:13 25 January 2011

Reaction of Amines with Resin-bound Benzoxazole-2-sulfonate

To a solution of 100 µl of diethyl- or di-n-propyl- or di-n-butylamine in 1.0 ml of methanol was added 1.0 g of resin tagged with benzoxazole-2-sulfonate. After warming in a water bath (60°C) for 5 min the solution was examined under UV light and analysed by TLC, HPLC and HPLC-MS. The procedure was repeated, with the mixture being kept at room temperature before chromatographic analysis. The procedure was also repeated using acetonitrile in place of methanol.

Reaction of Amino Acids with Benzoxazole-2-sulfonate

To 100 mg of glycine (or l-lysine or l-cysteine) dissolved in 1.0 ml of water was added a solution of 100 mg of sodium benzoxazole-2-sulfonate in 2.0 ml of water. The mixture was examined under UV light and then warmed in a water bath (60°C) for 5 min before being re-

examined under UV light. A portion of the reaction mixture was basified by dissolving about 200 mg of sodium bicarbonate in it and the solution re-examined under UV light.

Reaction of the amino acids with the resin-bound benzoxazole-2-sulfonate was also carried out as described above for the amines.

RESULTS AND DISCUSSION

The ideal derivatisation reagent for HPLC with UV or fluorescence detection should possess certain characteristics. Unlike a reagent such as dansyl chloride, its UV or fluorescence characteristics should be completely different from that of the derivative so that excess reagent would not interfere with the detection of the derivative. Alternatively, if the reagent and the derivative have similar spectra characteristics, their chromatographic behavior should be widely different to allow easy separation of the excess reagent from the derivative, if this cannot be achieved by simple solvent extraction. The reagent should react readily with the analyte, without any complicating side reactions, unlike reagents such as fluorescamine and NBD-chloride which sometimes undergo undesirable hydrolytic decomposition during derivatisation reactions with amines. The derivative should be stable, unlike the fluorescent derivatives of o-phthalaldehyde or fluorescamine with amines which are unstable to light. It should be possible to carry out the derivatisation reaction in a variety of solvents and solvent combinations that are likey to be encountered during the intended chromatographic applications. These are important requirements if the reagent is to be applicable to on-line pre- or post-column derivatisation

Downloaded At: 08:13 25 January 2011

in a possible automation of the analytical method. The reagent should be cheap, unlike a reagent such as fluorescamine.

Amines are fairly strong nucleophiles and the preparation of chromogenic derivatives of amines based on their reaction with reagents bearing sulfonate groups activated towards nucleophilic substitution has been reported. The most well-known reagents of this type are sodium 2,4-dinitrobenzene sulfonate [19-22] and sodium 2,6-dinitro-4-trifluoromethylbenzene sulfonate [23]. These reagents have the desirable property of being usable in aqueous media. The reagents, however, give non-fluorescent derivatives since the electron-withdrawing nitro groups required for the activation of the sulfonate moiety towards nucleophilic substitution are also strong inhibitors of fluorescence.

For the preparation of fluorescent derivatives of amines it was thought that a labile sulfonate group attached to a fluorescent aromatic nucleus would give a water-soluble reagent possessing the desirable characteristics outlined above.

Owing to the electron-withdrawing effect of the ring nitrogen the position alpha (or 2-) to the ring nitrogen is known to be one with a relatively high electron deficiency in the π-deficient N-heterocycles. A 2-sulfonate group in a N-heterocycle is, therefore, similar to that in arenesulfonates such as 2,4-dinitrobenzene sulfonate in its lability towards nucleophilic displacement. Because of the possible fluorescence of the heteroaromatic group, N-heterocycles with a 2-sulfonate group are promising candidates as ideal reagents for the derivatisation of amines and amino acids for HPLC-fluorescence analysis. Since benzoxazole is known to exhibit an intense blue fluorescence, sodium benzoxazole-2-sulfonate was

Downloaded At: 08:13 25 January 2011

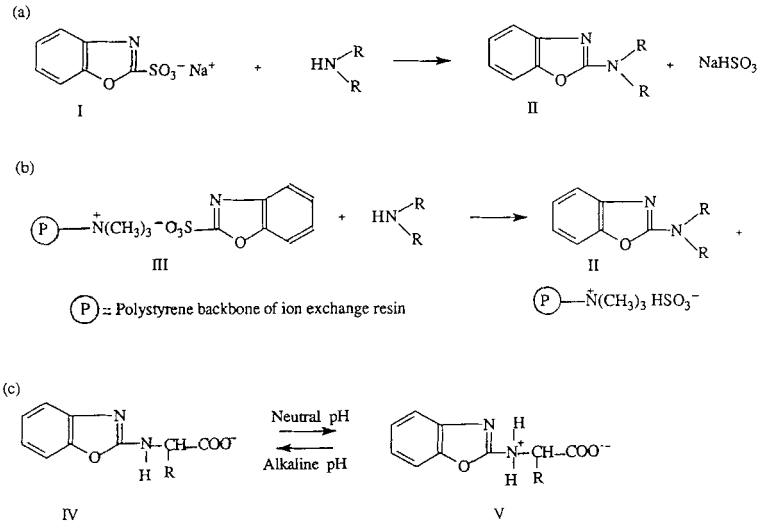


FIGURE 1. Illustration of (a): reaction of sodium benzoxazole-2-sulfonate with amines, (b): reaction of resin-bound benzoxazole-2-sulfonate with amines and (c) the ionic forms in which the benzoxazole derivatives of the amino acids may exist, depending on the pH.

Downloaded At: 08:13 25 January 2011

prepared and investigated as a possible example of such a reagent.

Preparation of Sodium Benzoxazole-2-sulfonate

Sodium benzoxazole-2-sulfonate has only been mentioned in an old patent literature [24]. It is readily obtained pure from 2-chlorobenzoxazole as described above. Although we had to prepare 2-chlorobenzoxazole, this compound is apparently available commercially (Aldrich Chemical Co).

Derivatisation of Amines and Amino Acids with Sodium Benzoxazole-2-sulfonate

The reaction of amines with sodium benzoxazole-2-sulfonate (I) is illustrated in figure 1(a). Formation

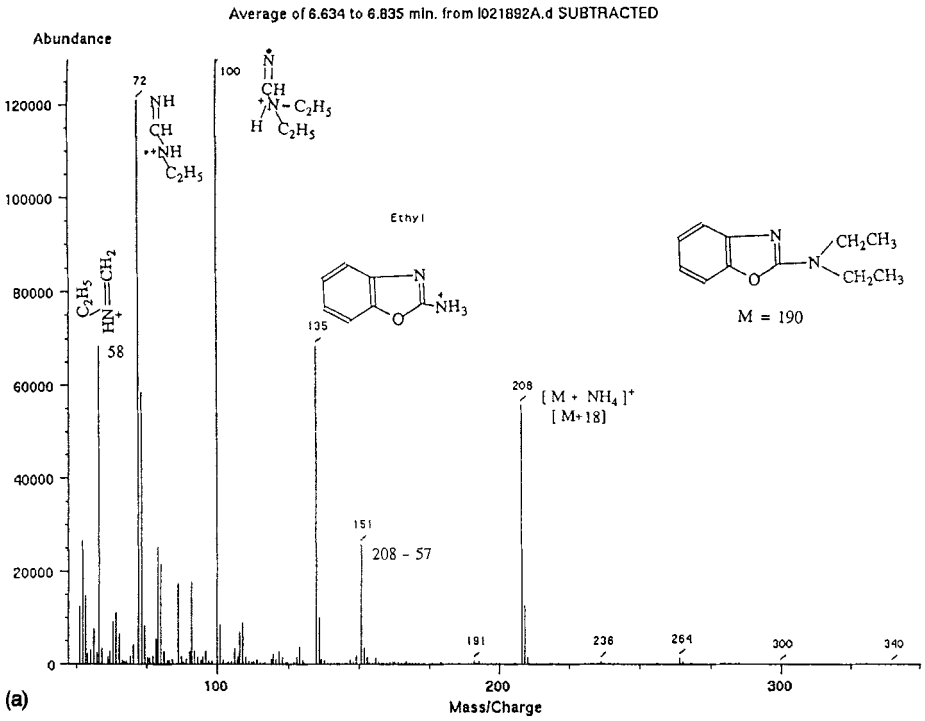
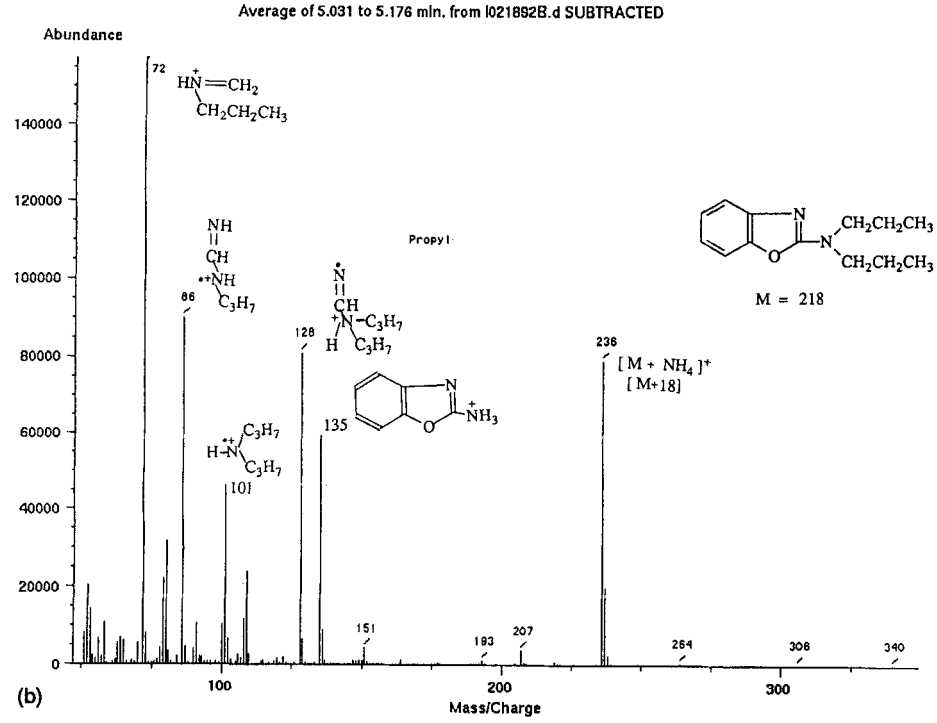


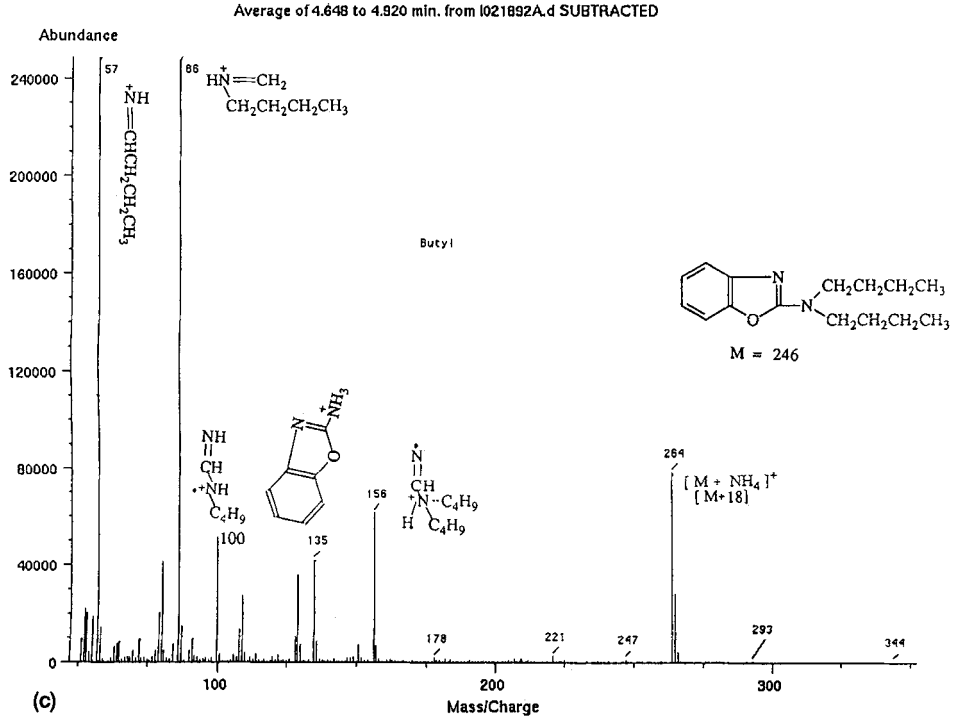
FIGURE 2.(a-c). Mass spectra of 2-(N,N-dialkylamino)benzoxazoles formed from the reaction of dialkyamines with benzoxazole-2-sulfonate.

of the derivatives was confirmed from their mass spectra The mass spectra of the derivatives of diethyl-, di-n-propyl- and di-n-butylamine are shown in figures 2a, 2b and 2c respectively. All the 2-(N,N-dialkyamino)benzoxazoles gave spectra which exhibit intense $[M+NH_4]^+$ ions due to the presence of ammonium acetate in the mobile phase used for the HPLC-MS system. The other peaks in the mass spectra of these compounds may be readily rationalised as shown in figure 3. The ion with m/z 135 is common to the mass spectra of 2-

Downloaded At: 08:13 25 January 2011

Downloaded At: 08:13 25 January 2011





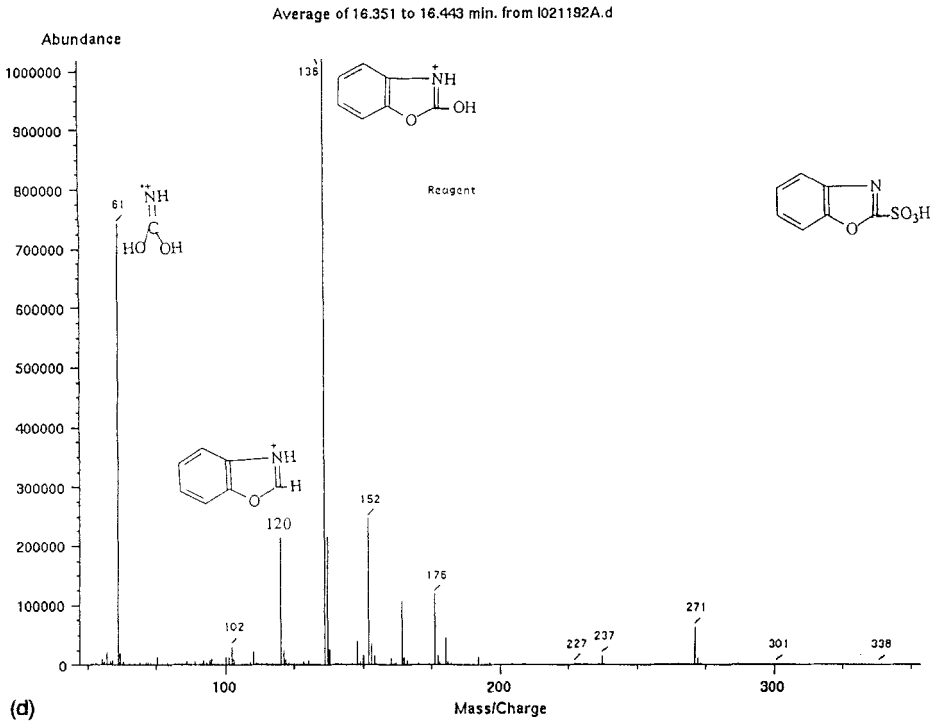


FIGURE 2d. Mass spectrum of benzoxazole-2-sulfonate

(N,N-dialkylamino)benzoxazoles and apparently results from loss of the two alkyl groups from the $[M+H]^+$ ion as the corresponding olefines. That is, loss of the alkyl groups accompanied by H-rearrangement. Fission of the bond between the O-atom and the 2-carbon atom is known to be the primary fragmentation step in the mass spectra of oxazoles [25]. This seems to be true for these 2-dialkyaminobenzoxazoles too as shown in figure 3. The apparent specificity of the fragmentation modes leading to the ions bearing the alkyl groups suggests that the benzoxazole derivatives may also be useful in the qualitative identification of unknown amines.

Downloaded At: 08:13 25 January 2011

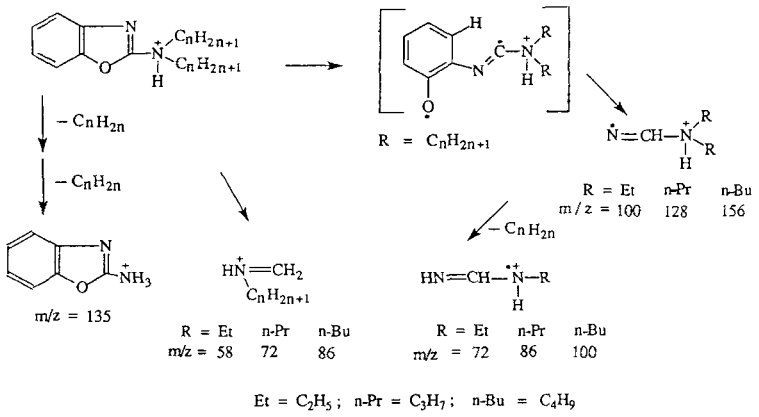


FIGURE 3. Rationalisation of the ion peaks in the mass spectra of 2-(N,N-dialkylamino)benzoxazoles

The mass spectrum of the reagent is shown in figure 2d. Apparently the base peak ion (m/z 136) results from elimination of SO_2 from the sulfonic acid.

Sodium benzoxazole-2-sulfonate was found to be non-fluorescent, probably because of the fluorescence inhibiting effect of the sulfonate group. The 2-(N,N-dialkylamino)benzoxazoles, however, exhibit an intense blue fluorescence when their solutions were observed under UV light. Reaction of the amines with sodium benzoxazole-2-sulfonate occurs rapidly, with the blue fluorescence of the 2-(N,N-dialkylamino)benzoxazoles appearing within a minute or two of adding an aqueous or methanol solution of the amine to an aqueous or methanol solution of the reagent. The derivatives were detectable at low microgram levels by TLC followed by examination of the plate under UV light. The rf of the derivatives of diethyl-, di-n-propyl- and di-n-butylamine were 0.60, 0.54, 0.51 respectively. Direct HPLC analysis of the

Downloaded At: 08:13 25 January 2011

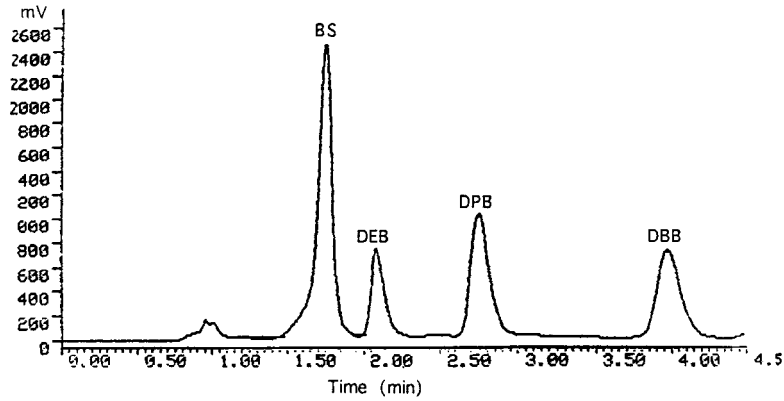


FIGURE 4. Chromatogram of a mixture of 2-(N,N-dialkylamino)benzoxazoles formed from the reaction of dialkylamines with benzoxazole-2-sulfonate.BS = Benzoxazole-2-sulfonate; DEB = 2-(N,N-diethylamino)benzoxazole; DPB = 2-(N,N-di-n-propylamino)benzoxazole; DBB = 2-(N,N-di-n-butylamino)benzoxazole.

Downloaded At: 08:13 25 January 2011

reaction mixture was found possible without interference from excess reagent which, being a salt, is poorly retained on the reversed phase column. A chromatogram of a reaction mixture of amines with sodium benzoxazole-2-sulfonate is shown in figure 4. If need be, the presence of excess reagent in the solution to be chromatographed may be avoided altogether by adding a little of a strong anion-exchange resin in the chloride form to the reaction mixture. Any excess reagent is sequestered or 'mopped up' by the resin. The derivatives may also be extracted with chloroform without interference from the reagent.

To examine the possibility of using the reagent for on-line pre- or post-column derivatisation of amines, the resin-bound form of the reagent was prepared as described. In this form the reagent still reacted as

readily with amines as when the reaction was carried out in a homogeneous medium. The reaction of amines with resin-bound benzoxazole-2-sulfonate is illustrated in figure 1(b). When the tagged resin was packed in a Pasteur pipet and a solution of the amines passed through the column of resin, the eluate exhibited the blue fluorescence of the 2-(N,N-dialkylamino)benzoxazoles.A chromatogram of an eluate containing a mixture of the derivatives is identical to that shown in figure 4 except that there is no peak due to excess reagent. The resin-bound benzoxazole-2-sulfonate would, therefore, be useful for on-line HPLC derivatisation of amines

Downloaded At: 08:13 25 January 2011

The inavailability of water-soluble reagents is a problem in the derivatisation of amino acids for HPLC -fluorescence analysis. Sodium benzoxazole-2-sulfonate is water-soluble, and was found to react with amino acids to give products which exhibit a blue flourescence under UV light. It was observed that the fluorescence of the reaction mixtures of the amino acids (except lysine) were not as intense as those of the aliphatic amines. This was thought to be due to the possibility that under the neutral reaction conditions some of the amino acid would exist as the zwitterion which would not react with benzoxazole-2-sulfonate, thereby lowering the yield of the fluorescent derivative. Furthermore, a proportion of the amino acid derivative itself would exist as the zwitterion (V) as illustrated in figure 1(c). The electron-withdrawing effect of the quarternary ammonium group in (V) would cause a lowering of the fluorescence of the derivative. These suggestions would explain why lysine, which has a second primary amino group, gave a reaction mixture which had a fluorescence intensity comparable to those of the reaction mixture of the dialkyamines. As expected, therefore, the fluorescence

of the reaction mixture of the amino acids was immediately intensified on dissolving some sodium bicarbonate in the mixture.

CONCLUSION

Benzoxazole-2-sulfonate may be considered an excellent reagent for homogeneous or heterogeneous derivatisation of amino compounds for HPLC-fluorescence (or UV) analysis. The principle described here seems quite versatile and may be extended to the use of other N-heteroaromatic 2-sulfonates. For example, sodium naphthoxazole-2-sulfonate was found to react with the amines too. But this compound was not considered as attractive as sodium benzoxazole-2-sulfonate because it is itself fluorescent and it is poorly soluble in water.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. J.O. Peggins III for the mass spectrometric analysis.

Downloaded At: 08:13 25 January 2011

REFERENCES

1. D.R. Knapp, Handbook of Analytical Derivatisation Reactions, John Wiley & Sons Inc., New York, 1979

2. J.F. Lawrence and R.W. Frei, Chemical Derivatisation in Liquid Chromatography, Elsevier, Amsterdam, 1976.

3. N. Seiler, Methods in Biochem. Anal., 18: 259 (1970).

4. N.E. Newton, K. Ohno and M.M. Abdel-Monem, J. Chromatogr., 124: 277 (1976)

5. M.M. Abdel-Monem and K.Ohno, J. Chromatogr., 107: 416 (1975)

6. S. Udenfriend, Pharmacology, 19: 223 (1979).

7. P.B. Ghosh and M.W. Whitehouse, Biochem. J., 108: 155 (1968)

8. F. VanHoof and A. Heyndrickx, Anal. Chem., 46: 286 (1974)

9. M. Roth, Anal. Chem., 42: 880 (1971)

10. R. Benson and P.E. Hare, Proc. Natl. Acad. Sci. (USA), 72: 619 (1975)

11. J.C. Hogkin, J. Liq. Chromatogr., 2: 1047 (1979)

12. S.C. Beale, J.C. Savage, D. Wiesler, S.M. Wietstock and M. Novotny, Anal. Chem., 60: 1765 (1988)

13. I.S. Krull, K.-H. Xie, S. Colgan, U. Neue, T. Izod,, R. King and B. Bidlingmeyer, J. Liq. Chromatogr., 6: 605 (1983)

14. K.-H. Xie, S. Colgan, I.S. Krull, J. Liq. Chromatogr. 6: (s-2), 125 (1983)

15. S.T. Colgan and I.S. Krull, in Reaction Detection in Liquid Chromatogrphy, I.S. Krull, ed., Marcel Dekker, Inc., New York, 1986, p 227

16. T.-Y. Chou, S.T. Colgan, D.M. Kao, I.S. Krull, C. Dorschel and B. Bidlingmeyer, J. Chromatogr., 367: 335 (1986)

17. T.-Y. Chou, C.-X. Gao S.T. Colgan, I.S. Krull, C. Dorschel and B. Bidlingmeyer, J. Chromatogr. 454: 169 (1983)

18. C.-X. Gao, I.S. Krull and T.M. Trainor, J. Chromatogr., 463: 192 (1989)

19. A.D. Smith and J.B. Jepson, Anal. Biochem., 18: 36 (1967)

20. D.J. Edwards and K. Blau, Anal. Biochem., 45: 387 (1972)

21. Y. Ishitoya, S. Baba and I. Hashimoto Clin. Chim. Acta, 46: 55 (1973)

22. S. Baba, I. Hashimoto and Y. Ishitoya, J. Chromatogr., 88: 373 (1974)

23. P.S. Doshi and D.J. Edwards, J Chromatogr., 176: 359 (1979)

24. Brit. Pat. 418291 (1934); Chem. Abstr. 29: 819 (1935)

25. H.-E. Audier, M. Fetizon, Y. Henry and T. Prange, Org. Mass Spect., 11: 1047 (1976)

Received: November 11, 1992
Accepted: November 30, 1992

Downloaded At: 08:13 25 January 2011