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Determination of 1-benzo[*b*]thien-2-ylethanone and related impurities by high performance liquid chromatography

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Abstract

1-Benzo[*b*]thien-2-ylethanone (2-acetylbenzothiophene, 2-ABT) and related impurities were determined using a reverse-phase high performance liquid chromatography system and UV detection at 254 nm. Separation was achieved isocratically on a 4.6 mm × 25 cm, 5 μm Zorbax Rx-C8 column using an eluent which is 0.2% perchloric acid/THF in a ratio of 60:40 (v/v). The chromatographic system resolved 2-ABT and known impurities in less than 45 min with near baseline resolution. Known impurities were quantitated versus 2-ABT with corrections made for differences in detector response at the specified wavelength. Linearity for 2-ABT was demonstrated with a correlation coefficient >0.9999. Assay precision (RSD values) for impurities at 0.5% ranged from ±1.8% to ±14%, while precision (RSD values) for the 2-ABT determination ranged from ±0.81% to ±1.1%. A variety of different chromatographic columns and conditions are discussed for the application.

Keywords: 2-Acetylbenzothiophene; Benzothiophenes; High performance liquid chromatography; 5-Lipoxygenase inhibitors

1. Introduction

Zileuton is a 5-lipoxygenase inhibitor currently in clinical trials for the treatment of asthma. A potential starting material in the synthesis of zileuton is 2-acetylbenzothiophene (2-ABT), which is chemically 1-benzo[*b*]thien-2-ylethanone (Fig. 1). Impurities contained in 2-ABT can either be carried through or react in the synthetic scheme of zileuton, forming impurities in the bulk drug substance. Therefore, it was important to develop a method that could determine 2-ABT and its potential impurities.

Capillary gas chromatography (GC) has been used for the determination of benzothiophenes in petroleum distillates [1–3]. A GC method has also been used for determining benzothiophenes in coal, oil shale and various environmental matrices [4–6]. Selective detection has been employed extensively due to the relatively low concentrations

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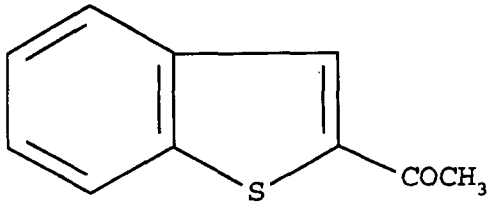


Fig. 1. Structure of 1-benzo[*b*]thien-2-ylethanone (2-ABT).

of the analytes and the complexity of the matrices. These selective detection techniques include sulfur chemiluminescence [7–10], radio frequency plasma detection [11], helium plasma optical spectroscopy [12] and mass spectroscopy [13–15].

The use of high performance liquid chromatography (HPLC) for substituted benzothiophenes and related polycyclic aromatic sulfur heterocyclic compounds has also been reported using various reverse- and normal-phase systems [16–18]. In our work, reverse-phase HPLC was chosen for determining 2-ABT because of its combination of speed, selectivity and simplicity. Additionally, since zileuton and its precursors are synthesized in aqueous and mixed-phase solvent systems, this technique had the advantage of being used for the determination of residual 2-ABT or related impurities at various steps in the synthesis process.

2. Experimental

2.1. Reagents and equipment

Acetonitrile and tetrahydrofuran (THF) were HPLC grade from EM Science (Cherry Hill, NJ). Deionized water used throughout this work was purified to $\geqslant$18 MΩ resistance with a Milli-Q system. Perchloric acid, 70% solution, was reagent grade from Fisher Scientific (Fair Lawn, NJ). An aqueous 0.2% perchloric acid solution was prepared by diluting 5 ml of 70% perchloric acid to 2 l with deionized water. The HPLC eluent was a mixture of an aqueous perchloric acid solution (0.2% v/v perchloric acid solution in water) and THF in a ratio of 60:40 (v/v). The 2-ABT standard and authentic related impurities were synthesized or isolated at Abbott Laboratories (North Chicago, IL). These materials were characterized by normal spectral, chromatographic and chemical techniques to assess their purity before use.

Chromatographic separations were achieved in the analytical method using a Zorbax Rx-C8 column (4.6 mm × 25 cm, 5 μm) from Mac-Mod Analytical (Chadds Ford, PA). The chromatograph used consisted of a SIL-9A autoinjector and a Model C-R4AX data system (Shimadzu, Kyoto, Japan) in combination with a Spectra-100 model UV detector and P-2000 chromatographic pump (Thermo Separation Products, Fremont, CA). Additional chromatographic columns evaluated for this separation included: Zorbax SB-CN (4.6 mm × 25 cm, 5 μm), Zorbax SB-Phenyl (4.6 mm × 25 cm, 5 μm) from Mac-Mod, Inertsil C-8 (4.6 mm × 25 cm, 5 μm) from MetaChem Technologies (Torrance, CA), Hypersil BDS C-8 (4.6 mm × 25 cm, 5 μm) and Lichrosorb RP-8 (4.6 mm × 25 cm, 5 μm) which were packed by Alltech Associates (Deerfield, IL).

2.2. Assay procedure and typical chromatographic conditions

For determination of impurities in 2-ABT an accurately weighed sample was dissolved in 10 ml of acetonitrile and diluted to 2.0 mg ml^{-1} in the HPLC eluent. Impurities were determined by comparison with a standard of 2-ABT prepared similarly and diluted to 0.01 mg ml^{-1} (representing 0.5%). For determinatin of 2-ABT, an accurately weighed sample or 2-ABT standard was dissolved in 10 ml of acetonitrile and then diluted to 0.10 mg ml^{-1} in the HPLC eluent. All determinations were performed by the external standard method by comparing integrated peak areas of impurities or 2-ABT with the appropriate standard. Known impurities were corrected for absorbance differences by applying appropriate response factors derived from authentic impurity standards, while unknown impurities were estimated versus 2-ABT.

Throughout this work, 20 μl injection volumes were used with a flow rate of 1.0 ml min^{-1}. The detector wavelength was at 254 nm using 0.05 and 0.2 absorbance units full scale respectively, for impurities and assay. UV detectors having spectral bandwidths of 6–8 nm were used. Isocratic conditions and ambient column temperature were used.

3. Results and discussion

2-ABT can be manufactured from substituted 2-thiobenzaldehydes such as 2-(methylthio)-benzaldehyde. These compounds are reacted with chloroacetone, followed by ring closure to produce the desired substituted benzothiophene. Alternatively, more hindered thiobenzaldehydes such as 2-(tert-butylthio)benzaldehyde can be converted to a disulfide intermediate followed by treatment with acetylacetone in a reverse aldol reaction scheme to produce 2-ABT. Both reaction schemes can potentially produce several manufacturing impurities. Presented in Fig. 2 are chromatograms showing 2-ABT which was spiked with known impurities and chromatographed using the conditions described in the assay procedure at three different detector wavelengths. The identities of the minor impurities are shown in Table 1. The parent 2-ABT has UV maxima at 204, 232 and 294 nm. As shown in Fig. 2, the detectability of the minor impurities is affected significantly by the choice of detector wavelength. A detector wavelength of 254 nm provided the most acceptable compromise of having both sensitivity and comparability of response for known minor impurities.

Large scale preparation and long term maintenance of reference standards for all known minor impurities is problematic in this application due to the instability of many of the thiobenzaldehydes and disulfides. Due to unavailabilty of sufficient amounts of reference compounds, quantitation of minor impurities was performed versus a 2-ABT standard preparation. Identification of minor impurities detected in the sample preparation was made using relative retention times versus 2-ABT. In order to correct for the difference in detector response between known impurities and 2-ABT, five-point calibration curves were constructed after chromatographing solutions of authentic characterized impurity standards and 2-ABT prepared in the concentration range 0.001 mg ml^{-1} to approximately 0.020 mg ml^{-1}. The chromatographic conditions used are stated in the text. A response factor was determined for each minor impurity as a ratio of its slope to that of 2-ABT. This factor is then applied to the calculation for the impurity on a weight percent basis. A plot of 2-ABT concentration (mg ml^{-1} x-axis) versus peak area (y-axis) gave a regression line of $y = -120 + (9.63 \times 10^3)x$ with a correlation coefficient >0.9999. Similar linearity was obtained for the known impurities. The relative retention times and calculated response factors for known minor impurities are included in Table 1.

3.1. Assay performance for minor impurities

In our initial attempts in developing a separation of 2-ABT and known impurities we used acetonitrile as the organic modifier. With this approach, 2-ABT, 3-hydroxyl-1-benzo(*b*)thien-2-ylethanone (peak 7) and 1-benzo(*b*)thien-2-yl(1,4-pentanedione) (peak 4) could not be resolved. Addition of THF to the acetonitrile modifier improved the resolution. However, gradient elution was necessary to elute (benzo(*b*)thien-2-oyl)benzo(*b*)thiophene (peak 10) in less than approximately 70 min while adequately retaining the 2-(methylthio)benzyl alcohol (peak 1). Use of neat THF as the modifier provided acceptable resolution of known impurities with the added advantage of eluting the most strongly retained impurities isocratically.

In routine use of the impurity method, the chromatographic performance is checked using a synthetic mixture containing 2-ABT spiked with approximately 0.5% each of 2,2′-dithiobenzaldehyde, 3-hydroxyl-1-benzo(*b*)thien-2-ylethanone, 2-(tert-butylthio)benzaldehyde and (benzo(*b*)thien-2-oyl)benzo(b)thiophene, which are respectively peaks 6, 7, 8 and 10 shown in Table 1 and Fig. 2. Prior to analysis the resolution factors, calculated according to USP [19], between 2-ABT and peak 6 (R_1) and between peaks 6 and 7 (R_2) are determined. Acceptable performance is indicated using the following criteria: $R_1 \geq 2.5$, $R_2 \geq 1.0$ and the retention time for peak 10 is ≤ 45 min. Slight variations in the aqueous/THF ratio were made and the above parameters were evaluated to determine the effects on the chromatography. These results are tabulated in Table 2. As shown by these data, a moderate decrease in the amount of THF modifier significantly lengthens the retention of peak 10 and decreases the amount of resolution

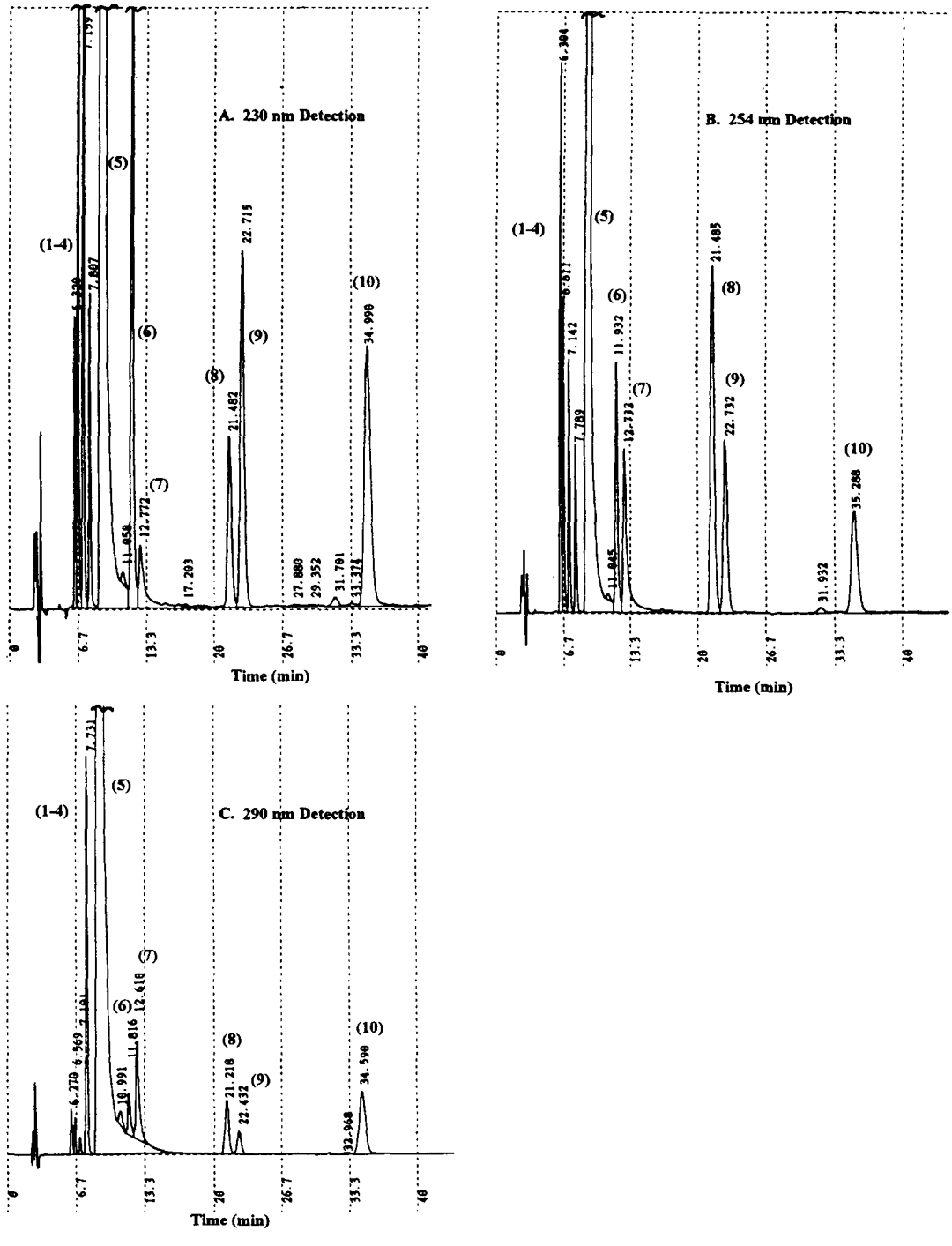
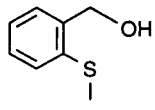
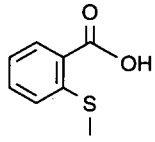
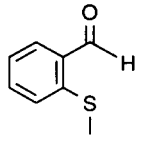
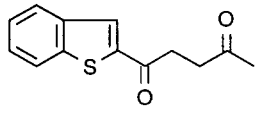
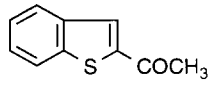
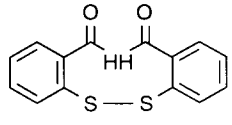
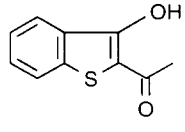
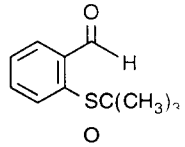
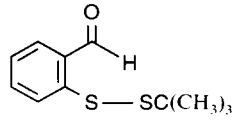
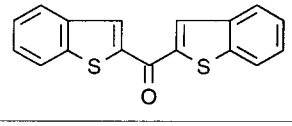
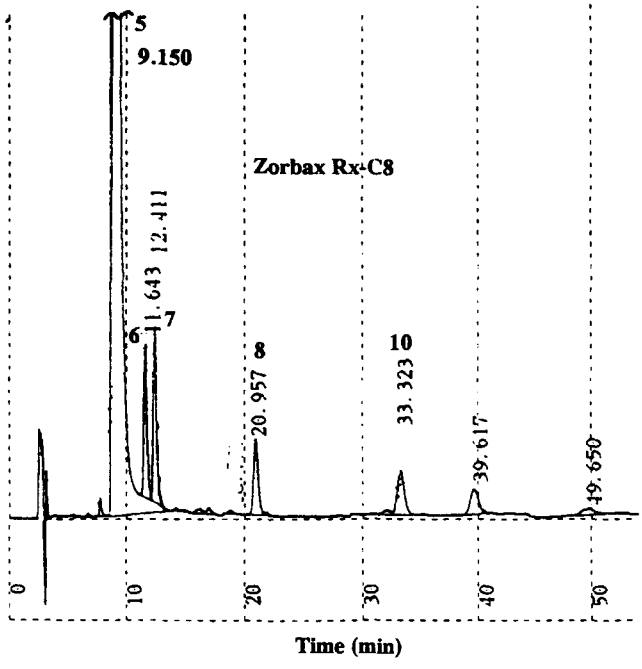


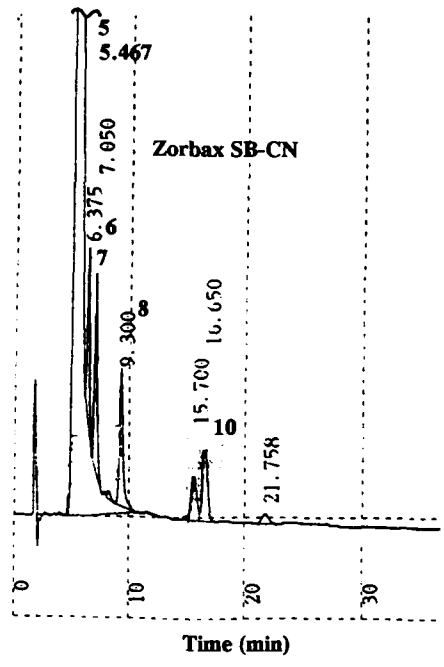
Fig. 2. Chromatograms of a synthetic mixture of 2-ABT and known impurities each at approximately 0.5%. Peak identities are shown in Table 1.

Table 1
Relative retention times (RRTs) and response factors (RFs) or potential manufacturing impurities in 2-ABT.

Peak	Structure	Name	RT	RF[a]
1		2-(Methylthio)benzyl alcohol	0.69	0.8
2		2-(Methylthio)benzoic acid	0.72	1.0
3		2-(Methylthio)benzaldehyde	0.78	1.2
4		1-Benzo(*b*)thien-2-yl(1,4-pentanedione)	0.86	1.2
5		1-Benzo(*b*)thien-2-ylethanone	1.00	1.0
6		2,2′-Dithiobenzaldehyde	1.3	0.8
7		3-Hydroxyl-1-benzo(*b*)thien-2-ylethanone	1.4	0.7
8		2-(tert-Butylthio)benzaldehyde	2.4	0.9
9		2-(tert-Butyldithio)benzaldehyde	2.5	1.4
10		(Benzo[*b*]thien-2-oyl)benzo[*b*]thiophene	3.9	1.0

[a]Response factor calculated at 254 nm.





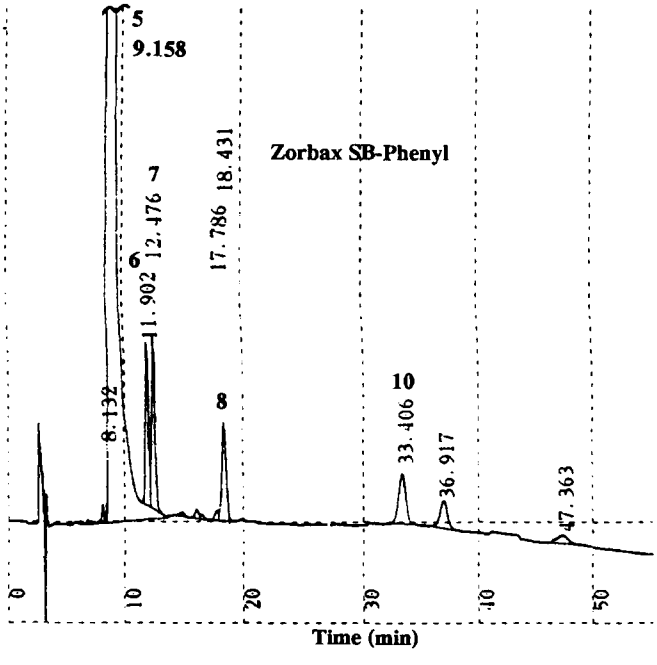
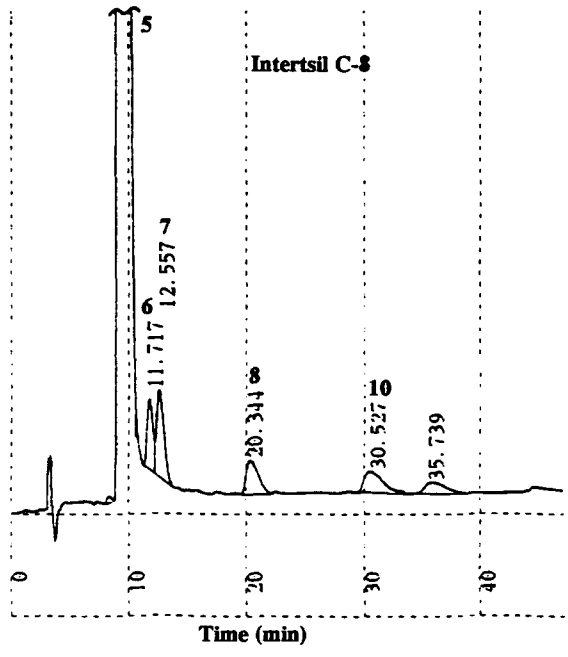
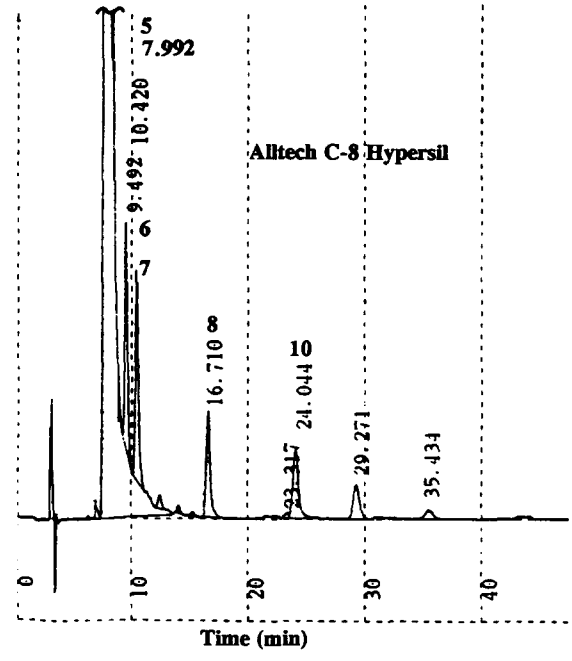


Fig. 3. Comparative chromatograms of a synthetic mixture of 2-ABT and known impurities. Peak identities are shown in Table 1.





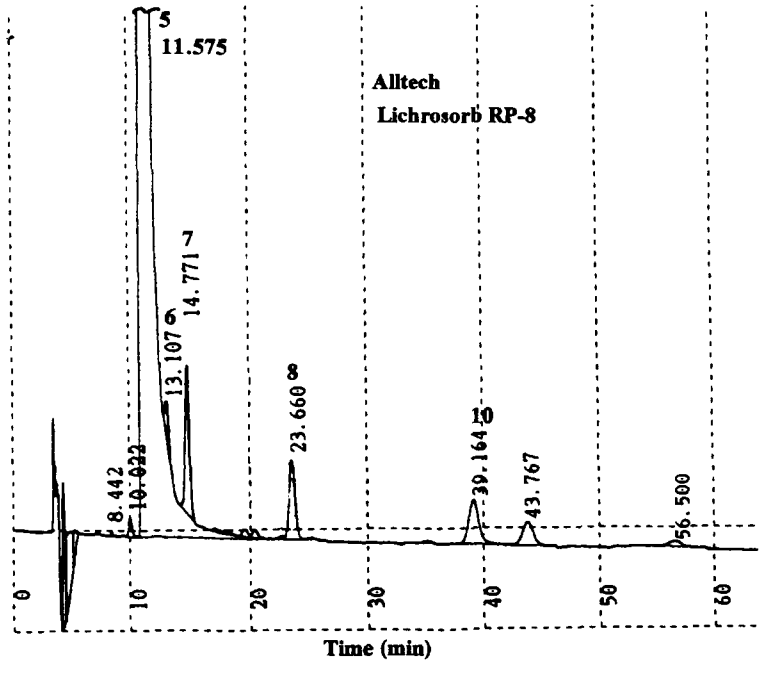


Fig. 4. Comparative chromatograms of a synthetic mixture of 2-ABT and known impurities. Peak identities are shown in Table 1.

Table 2
Chromatographic performance at various levels of THF modifier

% THF	R_1[a] (2-ABT/peak 6)	R_2[a] (peak 6/peak 7)	RT (min) of peak 10
45	2.4	2.2	20.0
40	5.0	1.4	36.0
35	8.6	0.83	>65

[a]Resolution factors calculated according to USP for pairs of peaks identified in Table 1.

between peaks 6 and 7. Increasing the amount of THF elutes all peaks faster at the expense of the 2-ABT and peak 6 resolutions. The criteria were met after extended use in our laboratory on several columns which were manufactured from different batches of packing. Variations of the $HClO_4$ concentration used in the eluent preparation from 0.1% to 0.5% resulted in minimal changes in resolution and retention times of all peaks. Elevating the column temperature to 35°C eluted all peaks faster, but did not significantly improve peak resolution or symmetry for the minor impurities.

Precision data for the impurity determination were obtained from the synthetic sample described above. Determinations were performed by two analysts on separate days using different columns and equipment. These data are summarized in Table 3 and, as shown, RSD values ranged from ±1.8% to ±14%. Several of the impurities contained in this synthetic mixture were identified in early authentic lots of 2-ABT. The fate of these compounds varies as the starting 2-ABT is converted to the final bulk drug substance. Their presence at low levels in advanced intermediates or in the bulk drug substance was observed in early synthetic support. Significant process refinements have since been made in the synthesis of 2-ABT. Current lots of this material contain greatly reduced amounts of the impurities.

The impurity assay was evaluated on several alternate reverse-phase columns and compared with the Zorbax Rx-C8 packing described in the method. The synthetic mixture used previously was initially chromatographed using the same conditions on Zorbax SB-CN and Zorbax SB-Phenyl columns. As shown in Fig. 3, comparable retention of 2-ABT was obtained for the phenyl packing. However, the resolution of 2,2′-dithiobenzaldehyde (peak 6) and 3-hydroxy-1-benzo(*b*)thien-2-ylethanone (peak 7) was significantly less than on the Zorbax Rx-C8. Appreciably shorter retention times were obtained on the cyano phase. Additional reverse-phase C-8 and C-18 phases were also evaluated. As shown in Fig. 4, less acceptable results were obtained.

3.2. Assay performance for 2-ABT determination

Typical chromatograms for the 2-ABT determination are shown in Fig. 5. Three bulk drug lots of 2-ABT were determined on an "as is" basis by two

Table 3
Precision data for 2-ABT impurities

Day	Analyst	% 2,2′-Dithiobenzaldehyde	% 3-Hydroxyl-1-benzo(*b*)thien-2-ylethanone	% 2-(tert-Butylthio)benzaldehyde	% (Benzo[*b*]-thien-2-oyl) benzo[*b*]thiophene
1	1	0.46	0.59	0.43	0.43
1	1	0.43	0.61	0.43	0.46
1	1	0.39	0.63	0.45	0.49
2	2	0.44	0.57	0.44	0.39
2	2	0.40	0.57	0.43	0.35
2	2	0.56	0.57	0.44	0.36
Mean		0.45	0.59	0.44	0.41
SD±		0.061	0.025	0.008	0.056
RSD±		14%	4.2%	1.8%	14%

Table 4
Precision data for 2-ABT determinations, % 2-ABT "as is" basis

Day	Analyst	Lot 1	Lot 2	Lot 3
1	1	99.7	100.2	100.2
1	1	96.8	99.9	100.1
1	1	98.6	99.2	99.9
2	2	98.7	98.4	99.8
2	2	97.6	98.4	98.5
2	2	99.1	97.3	98.4
Mean		98.4	98.9	99.5
SD±		1.05	1.08	0.81
RSD±		1.1%	1.1%	0.81%

analysts on separate days using different columns and equipment. These data, presented in Table 4, showed precision (RSD values) ranging from ±0.81% to ±1.1%.

4. Conclusion

This work has presented a fast, reliable and rugged assay for 2-ABT and associated minor impurities. The determinations are based on an isocratic HPLC separation using a simple binary mixture of dilute perchloric acid and THF. Additional columns and conditions were discussed and compared with those described in the method.

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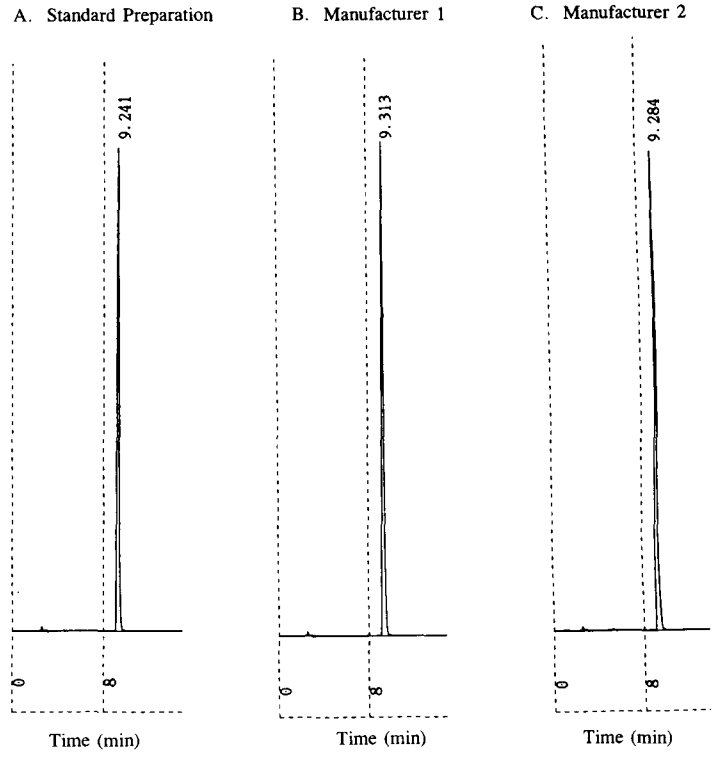

Fig. 5. Typical chromatograms for 2-ABT determination.

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