Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 63–74
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Utilizing capillary gas chromatography mass spectrometry to determine 4-benzotrifluoride *t*-butyl ether as a reaction by-product in fluoxetine synthesized using potassium *t*-butoxide as base

David K. Robbins *, Paul N. Dodson, Lynne A. Buccilli, David Mitchell

Lilly Research Laboratories, Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Received 11 October 2001; received in revised form 9 September 2002; accepted 16 September 2002

Abstract

Fluoxetine hydrochloride has been prepared using two similar synthetic routes, both of which rely upon an ether formation reaction mediated by a base. The base used can affect the impurity profile of this reaction. It was proposed that the synthesis of fluoxetine carried out using potassium *t*-butoxide as base and 4-chlorobenzotrifluoride (or 4-fluorobenzotrifluoride) in the ether formation step may result in the formation of 4-benzotrifluoride *t*-butyl ether as a reaction by-product. To test this hypothesis, capillary gas chromatography-mass spectrometry (GC/MS) was utilized to evaluate samples of free base fluoxetine synthesized using sodium hydride (NaH) or potassium *t*-butoxide as the base. Assay conditions using selected ion monitoring (SIM) were developed, which allowed detection of trace levels (parts per million, ppm) of 4-benzotrifluoride *t*-butyl ether in fluoxetine free base sample matrix. Response linearity, precision, and standard spike recovery were examined during development, and were found to be suitable. Comparisons of fluoxetine samples generated from both NaH and potassium *t*-butoxide processes were performed using the GC/MS assay.

Keywords: 4-Benzotrifluoride *t*-butyl ether; Fluoxetine; Potassium *t*-butoxide; Gas chromatography; Mass spectrometry

1. Introduction

Fluoxetine hydrochloride (Prozac®) was the first major introduction of the Selective Serotonin Reuptake Inhibitor (SSRI) drugs for clinical depression [1–5]. In the manufacturing of this important therapeutic, one commonly used reaction is an ether formation mediated by an alkoxide or phenoxide. Various bases, including sodium hydride (NaH) and potassium *t*-butoxide, have been utilized in the manufacturing process [6–11]. The choice of base used does have a potential impact on the impurity profile.

* Corresponding author. Tel.: +1-317-276-9625; fax: +1-317-276-4507.
E-mail address: dkrobbins@lilly.com (D.K. Robbins).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00605-2

The synthesis of fluoxetine (**I**) using potassium *t*-butoxide as base in the ether forming step, could result in the formation of 4-benzotrifluoride *t*-butyl ether (**II**) as a reaction by-product [12–18]. A comparison of the ether-forming steps from both the NaH and potassium *t*-butoxide routes are shown in Fig. 1. Although most nucleophilic aromatic substitution (SNAR) routes to fluoxetine are similar, this impurity can only occur with the potassium *t*-butoxide process. Although not nucleophilic, when potassium *t*-butoxide (**III**) is used as the base, it can potentially react directly with 4-chlorobenzotrifluoride (PCBT), (**IV**), resulting in the formation of 4-benzotrifluoride *t*-butyl ether (**II**) [19]. However, when NaH is used as the base in the ether forming step (as in the synthesis of Prozac®), hydrogen gas (H_2) is liberated, which does not react with the aryl halide.

Assay conditions capable of detecting relatively low levels of 4-benzotrifluoride *t*-butyl ether in a complex sample matrix were required in order to study impurity profiles of fluoxetine synthesized using the two different bases. Numerous impurities of fluoxetine, resulting from various synthetic routes, have been evaluated previously using both HPLC and capillary GC techniques [20,21]. Since 4-benzotrifluoride *t*-butyl ether is a neutral molecule, it is a poor candidate for the atmospheric pressure ionization techniques (electrospray and atmospheric pressure chemical ionization) available in LC/MS. Capillary GC/MS offered both specificity and sensitivity with electron impact (EI) ionization and was chosen to evaluate the various crude reaction samples with the option of employing selected ion monitoring (SIM) for detecting trace levels if needed.

2. Experimental

2.1. Solvents and chemicals

Chromatographic grade methylene chloride was obtained from Burdick & Jackson (Muskegon, Michigan, USA). Quantities of authentic 4-benzotrifluoride *t*-butyl ether were synthesized for use as a standard, the authenticity of which was confirmed by proton NMR and GC/MS (Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA) [22]. 4-Trifluoromethylphenol, 99%$^+$, was obtained from Sigma–Aldrich (Milwaukee, Wisconsin, USA).

2.2. Equipment

Capillary gas chromatography-mass spectrometry (GC/MS) with EI source was used for evaluations of reaction products. The GC/MS system

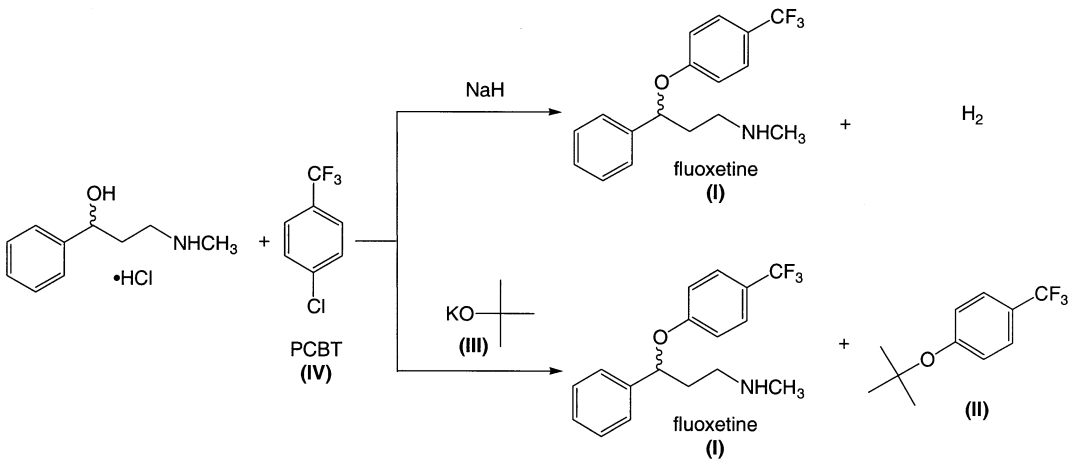

Fig. 1. A comparison of the ether forming step in the synthesis of fluoxetine. 4-Benzotrifluoride *t*-butyl ether (**II**, *m/z* 218.2) is a potential impurity of fluoxetine (**I**) when the synthesis is done using potassium *t*-butoxide as base.

used was a Hewlett–Packard model 5972 MSD coupled to a HP5980 Series II Plus GC equipped with both split/splitless and cool on-column injectors, operated using an MS ChemStation data system (G1701BA, Agilent Technologies, Inc., Wilmington, Delaware, USA). The columns used for these investigations were (a): DB-1 (30 m × 0.25 mm id, 0.25 μm film thickness, 100% dimethylpolysiloxane, J&W Scientific, Folsom, California, USA), (b) DB-1701 (30 m × 0.25 mm id × 0.25 μm film thickness, (14% cyanopropylphenyl)-methylpolysiloxane, J&W Scientific, Folsom). A 1 m × 0.53 mm id deactivated fused silica retention gap was attached to the injector end of each of the analytical columns as a guard column (J&W Scientific, Folsom).

When used in the full scan mode, the MS was set to scan from *m*/*z* 33 to 350. Ions monitored when operating in the SIM mode were *m*/*z* 218, 203, and 162. The column temperature program for use with the DB-1 was as follows: 40 °C, hold for 1 min, then ramp at 15 °C/min to 300 °C, hold for 10 min. Sample injections of 1 μl were used, split at 1:25, with the injector at 250 °C. For assays using the cool on-column injection technique with a DB-1701, the oven program was the following: 40 °C, hold for 1 min, then ramp to 280 °C at 15 °C/min. Hold at 280 °C for 10 min. Helium was used as carrier gas for all experiments, with the inlet pressure set to 49.0 kPa (36 cm/s linear flow).

2.3. Sample and standard solution preparation

2.3.1. For qualitative GC/MS investigation

Using a Mettler MT5 microbalance, 2–15 mg of reaction samples were weighed directly into GC autosampler vials. Samples were dissolved in 1 ml methylene chloride.

2.3.2. Linearity standard preparation

Approximately 20 mg of 4-benzotrifluoride *t*-butyl ether was weighed accurately into a 10 ml volumetric flask. The flask was diluted to volume with methylene chloride. Using this stock solution, a set of serial dilutions was then prepared with concentrations ranging from approximately 0.2 to 20 μg/ml.

2.3.3. Standard spike recovery

Triplicate sample solutions were prepared in the following fashion: From the standard stock solution above, 0.1 ml was transferred into a 10 ml flask and diluted to volume with methylene chloride. From this solution, 0.1 ml was transfered into a 5 ml flask and diluted to volume with methylene chloride. The final concentration was approximately 0.4 μg/ml. Approximately 25 mg of fluoxetine, from the NaH synthetic process, was weighed into a 1 ml flask. The fluoxetine was then diluted to volume with the 0.4 μg/ml standard solution. The resulting standard spike was equivalent to approximately 16 ppm versus the total sample concentration.

2.3.4. Precision evaluation

A sample containing approximately 800 ppm 4-benzotrifluoride *t*-butyl ether was prepared in triplicate by dissolving and diluting 25 mg into 1 ml of methylene chloride.

3. Results and discussion

3.1. GC/MS Investigations of impurity identity

A methylene chloride solution of authentic 4-benzotrifluoride *t*-butyl ether (**II**) was analyzed by capillary GC/MS to evaluate its' chromatographic behavior and to generate an EI spectra. The results of this initial investigation, using a DB-1 column, can be seen in Fig. 2. The molecular ion at *m*/*z* 218 can be seen to be very weak, as is *m*/*z* 203. The most abundant fragment, *m*/*z* 162, is the result of a hydrogen rearrangement.

A sample of the free base of fluoxetine (**I**) was synthesized using potassium *t*-butoxide as the base in the arylation step as shown in Fig. 1. The resulting material was then evaluated using the same GC/MS conditions as those for the 4-benzotrifluoride *t*-butyl ether standard used previously in Fig. 2. The total ion chromatogram (TIC) and spectra, shown in Fig. 3, provided verification that 4-benzotrifluoride *t*-butyl ether (**II**), was present as by-product of the potassium *t*-butoxide process. A peak matching the retention time of the 4-benzotrifluoride *t*-butyl ether stan-

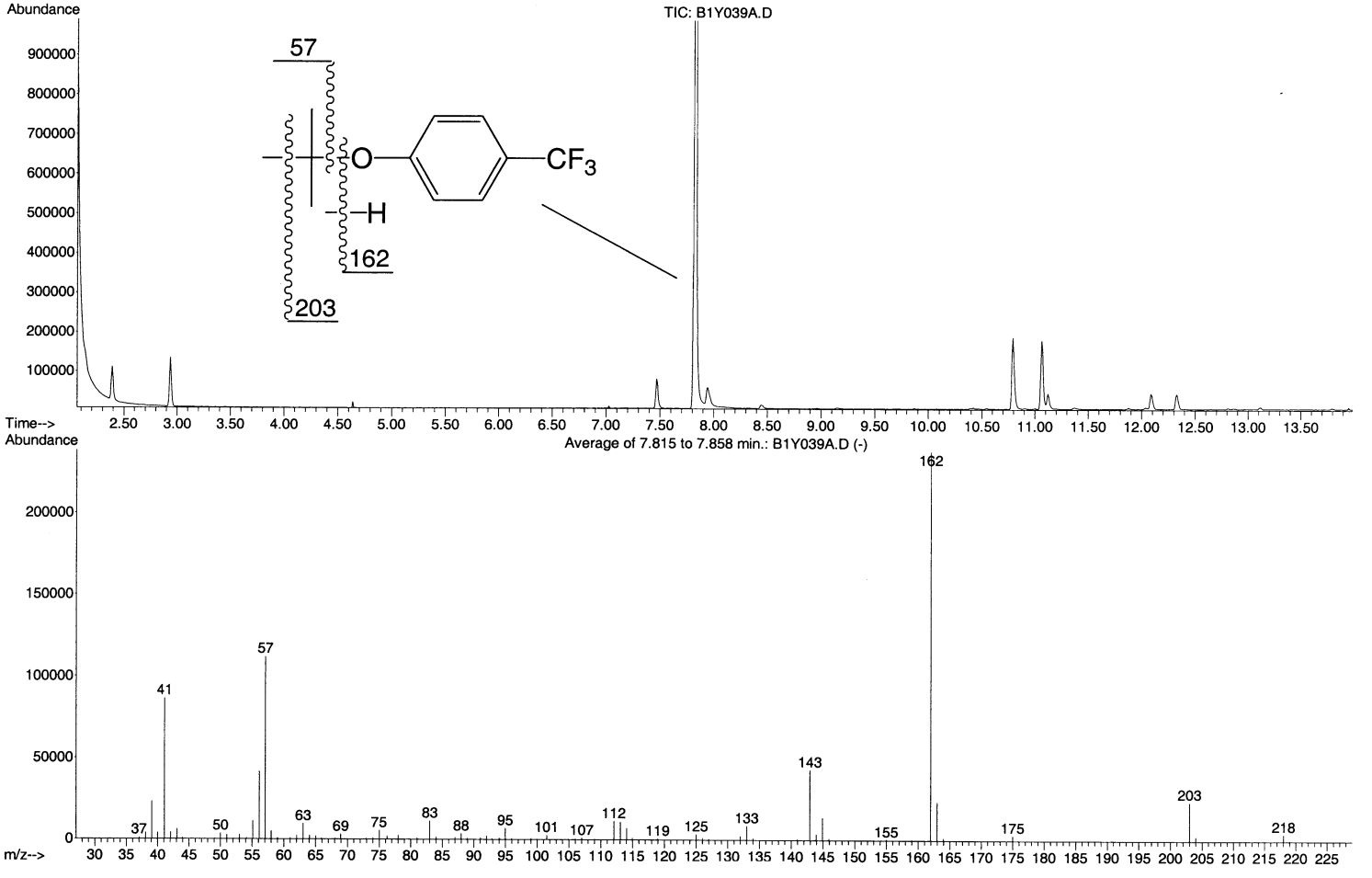

Fig. 2. The TIC and EI spectra for isolated 4-benzotrifluoride *t*-butyl ether, *m/z* 218.2.

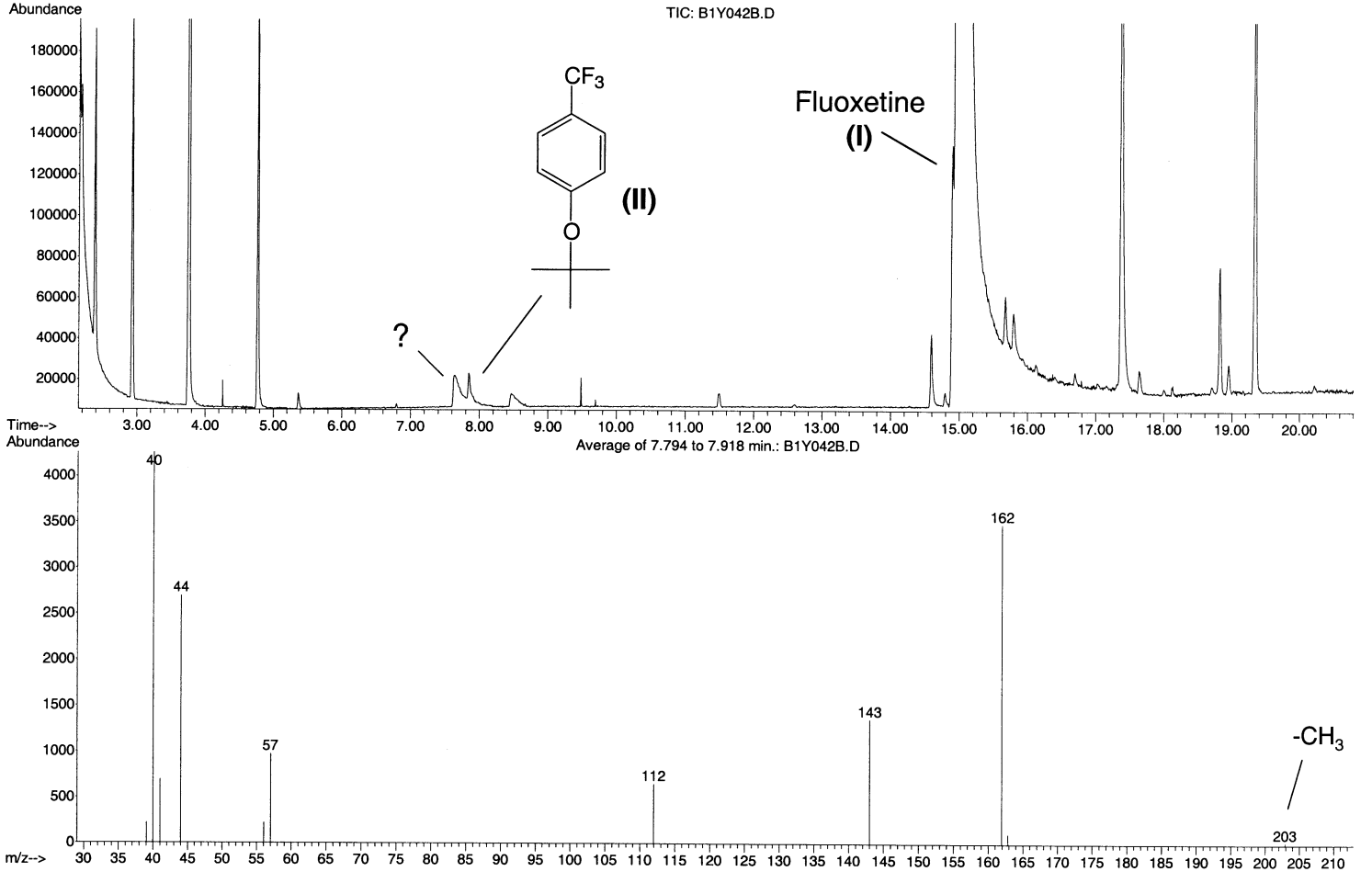

Fig. 3. TIC and EI spectra of fluoxetine prepared via the potassium *t*-butoxide synthesis, provided verification that 4-benzotrifluoride *t*-butyl ether was a by-product. The relative abundance of *m*/*z* 218 was too low to detect on this chromatogram.

dard was detected in the fluoxetine sample. It was noted that although the *m*/*z* 218 abundance was too weak to be detected in the impurity peak, the pattern closely matched the standard spectra seen in Fig. 2. The *m*/*z* 57 fragment indicated the loss of a *tert*-butyl group, only possible with 4-benzotrifluoride *t*-butyl ether (**II**).

As seen in Fig. 3, the peak for (**II**), eluted on the tail of another poorly shaped impurity peak. The resulting EI spectra indicated the tailing peak had a structure closely related to the 4-benzotrifluoride *t*-butyl ether. The spectra led to the proposal that the early eluting impurity was the phenolic analog, 4-trifluoromethylphenol. To verify this, a qualitative mixture of authentic phenol (**V**) and (**II**) was prepared and analyzed. The identity of the tailing peak was then confirmed as the phenol (**V**), Fig. 4.

Cool on-column injection was employed to minimize potential formation of 4-trifluoromethylphenol through thermal degradation of 4-benzotrifluoride *t*-butylether or fluoxetine in the high temperature split injection port (250 °C). Fluoxetine (**I**) from the potassium *t*-butoxide process was assayed using the cool on-column injection technique, rather than the heated split injection method used previously. The injector port temperature was set at 40 °C for the cool on-column injection method, the same as the initial oven temperature. The oven program used was the same as for the split injection method described previously. Both (**II**), and (**V**), were detected using cool on-column injection at levels similar to those seen with split injection. These results indicated that 4-benzotrifluorophenol was already present in the fluoxetine sample as a reaction by-product and not due to thermal degradation occurring in the split injection port [21].

3.2. Development of quantitative assay conditions for detection of 4-benzotrifluoride-t-butyl ether

For detection of low levels (i.e. ppm) of 4-benzotrifluoride *t*-butyl ether (**II**) in samples of fluoxetine (**I**), GC/MS operating in the SIM mode was the technique of choice. The SIM technique allows the mass spectrometer to be tuned to detect only specific ions (fragments) unique to the compound of interest. The advantage is that all other ions, other than those selected, are not detected, usually eliminating interfering peaks and background signals. For maximum sensitivity, the most desirable ions are the most abundant ions unique to the selected molecule. In this case, the *m*/*z* 218 and 203 ions were unique to 4-benzotrifluoride *t*-butyl ether (**II**) and were very low in relative abundance.

Cool on-column injection allowed much higher sample loading, therefore, increasing the relative abundance of *m*/*z* 218 and *m*/*z* 203 ions for use with SIM. Due to peak overlap when using the DB-1, it was difficult to distinguish between 4-benzotrifluoride *t*-butyl ether (**II**) and 4-trifluoromethylphenol (**V**) when the molecular ion (*m*/*z* 218) and the first loss (*m*/*z* 203) were too low to detect. Improving the separation of the *t*-butyl ether (**II**) and phenol (**V**) impurities was then investigated.

It was determined that the midrange polarity DB-1701 improved resolution dramatically, actually reversing elution order of (**II**) and (**V**). Using the DB-1701 phase, (**II**) eluted before the more polar phenol (**V**), with greater than 1 min separation. An example chromatogram of a qualitative mixture of both 4-benzotrifluoride *t*-butyl ether (**II**) and 4-benzotrifluorophenol (**V**) analyzed using DB-1701 is shown in Fig. 5. Improved resolution eliminated ion contribution from (**V**) to the signal for 4-benzotrifluoride *t*-butyl ether (**II**) due to co-elution. Therefore, the separation resulting from using the DB-1701 allowed use of the *m*/*z* 162 ion as well as both *m*/*z* 218 and *m*/*z* 203 ions, improving sensitivity significantly for the ether (**II**).

GC/MS, operating in the SIM mode (*m*/*z* 218, 203, 162) with a DB1701 and cool on-column injection, was used to evaluate samples of fluoxetine (**I**) which were from both the NaH and potassium *t*-butoxide processes. Examples of TICs from the comparison are shown in Fig. 6. These were matched against a solution containing both 4-benzotrifluoride *t*-butyl ether (**II**) and 4-benzotrifluorophenol (**V**) in methylene chloride. The 4-benzotrifluoride *t*-butyl ether (**II**) is clearly visible only in the sample synthesized using potassium *t*-butoxide (**III**).

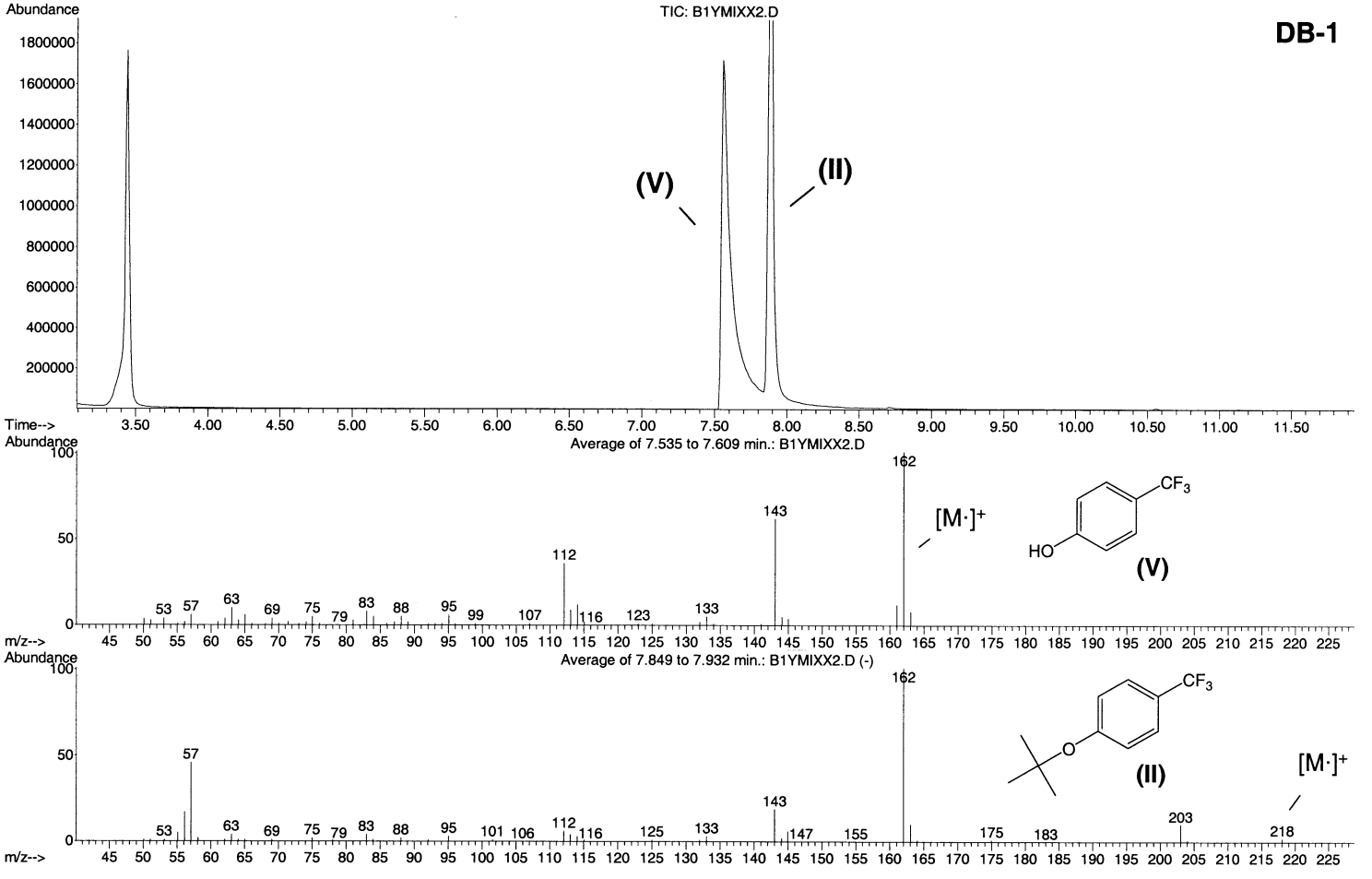

Fig. 4. TIC and EI spectra of 4-benzotrifluorophenol (**V**) and 4-benzotrifluoride *t*-butyl ether (**II**).

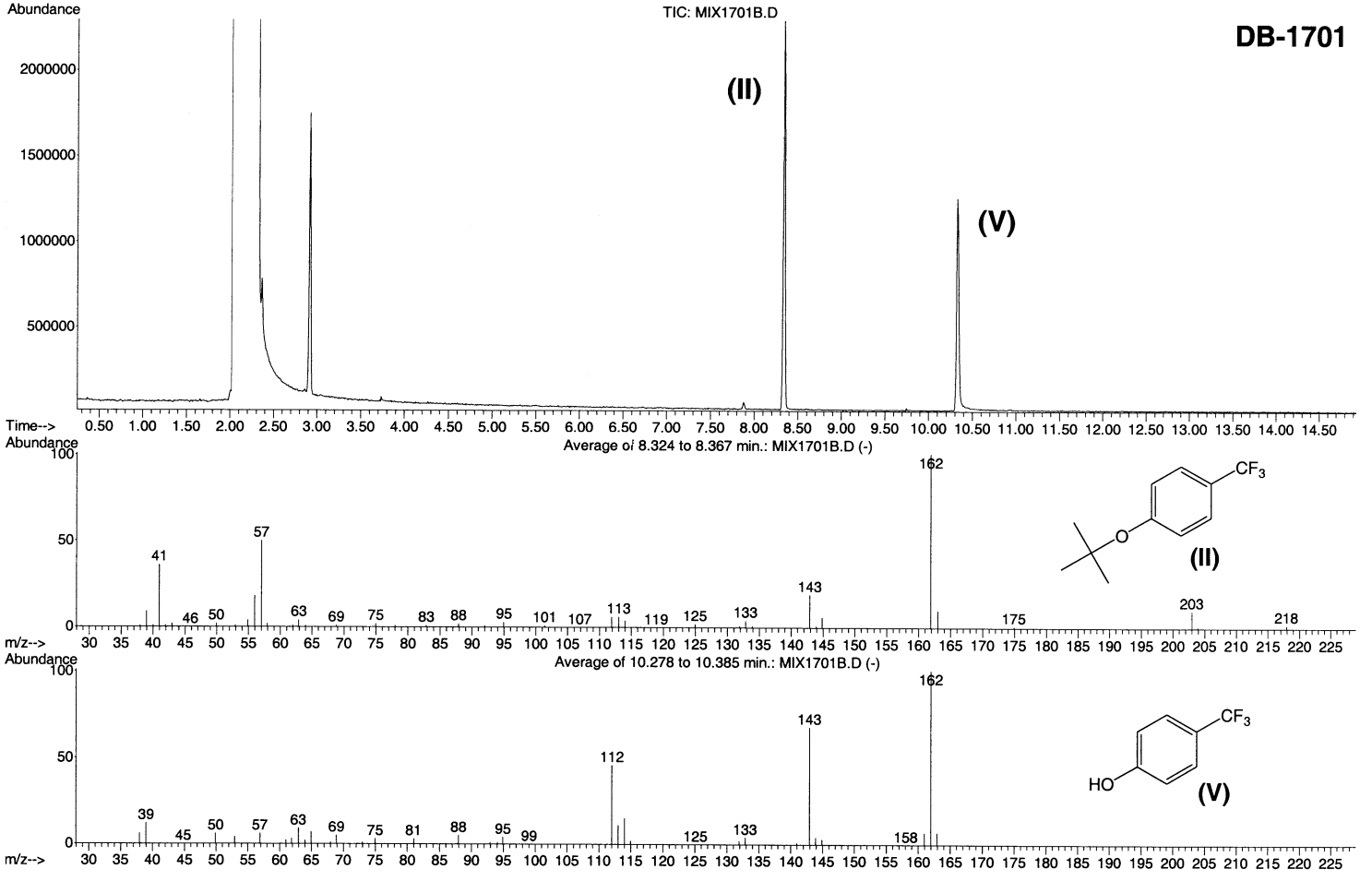

Fig. 5. The improved resolution eliminated signal contribution from the phenol (**V**) due to co-elution and allowed use of *m/z* 162 as well as *m/z* 218 and *m/z* 203, improving sensitivity significantly for the *t*-butyl ether (**II**).

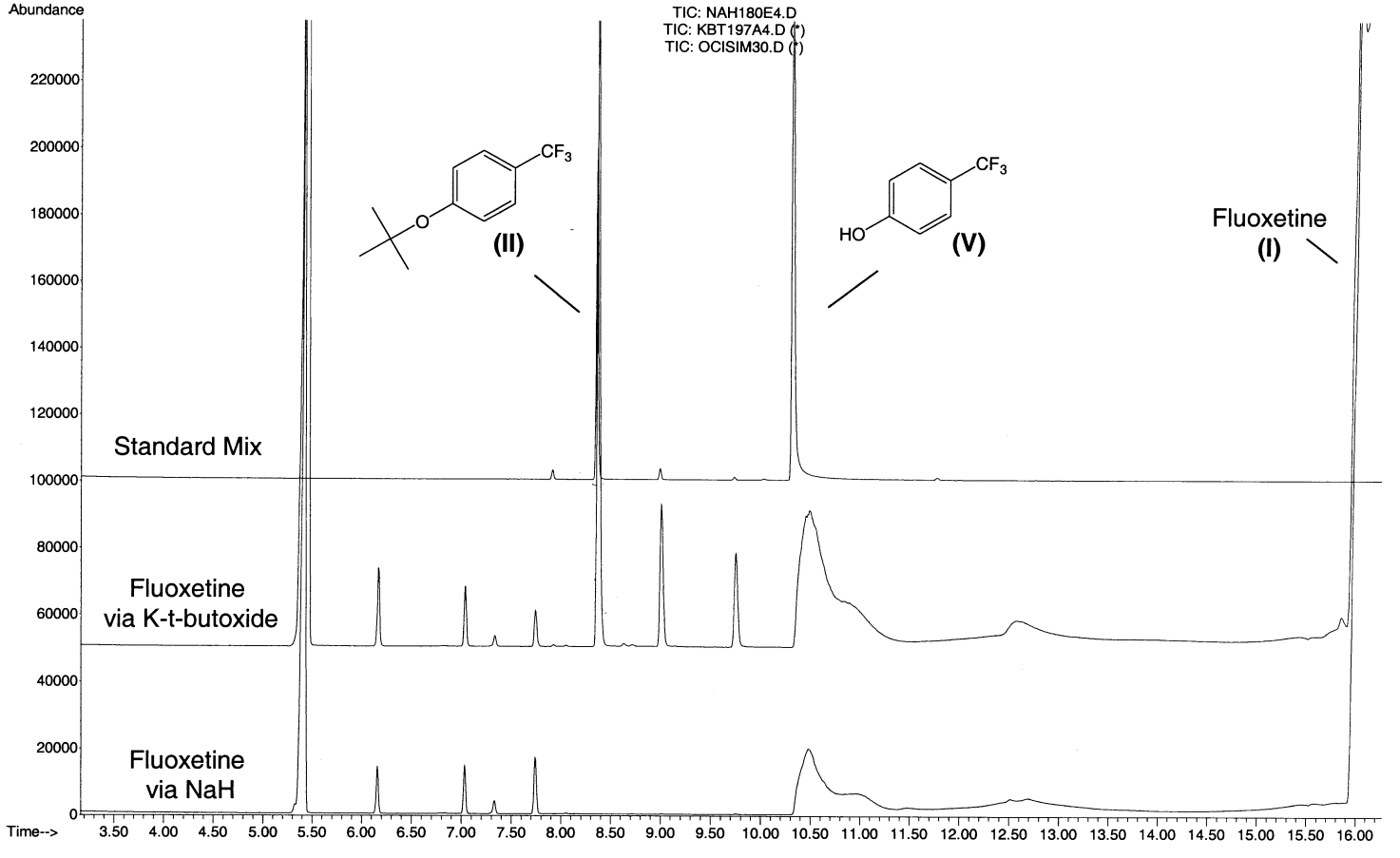

Fig. 6. Comparison of samples of fluoxetine from the NaH and potassium *t*-butoxide processes versus a standard of 4-benzotrifluoride *t*-butyl ether.

In order to quantify 4-benzotrifluoride *t*-butyl ether (**II**) in fluoxetine (**I**) using the GC/MS SIM assay technique, a series of standard solutions were assayed to determine the linearity of the standard concentrations versus area responses. The evaluation of the standard solutions indicated a linear response over a broad range, with a linear least squares regression represented by the equation: $Y = -63\,908.2 + 243\,671.8X$, where Y represents area response and X is the concentration. Correlation between the relative area response and standard concentration was very good at 0.9999 over the concentration range studied. The signal to noise ratio was excellent, and was determined to be nearly 100–1, using the corrected signal of lowest standard peak (0.16 μg/ml) versus baseline RMS noise.

To evaluate efficiency of the assay, a standard addition spike recovery study was performed. For this study, three solutions of fluoxetine from the NaH process were prepared at similar concentrations, by dilution with a standard solution consisting of 0.32 μg/ml of 4-benzotrifluoride *t*-butyl ether (**II**) in methylene chloride. Sample concentrations of fluoxetine were between 24.0 and 27.5 mg/ml. Therefore, the 4-benzotrifluoride *t*-butyl ether (**II**) spike added to each sample, was equivalent to between 11.6 and 12.9 ppm versus the total fluoxetine sample concentration. These standard spike peaks were easily detected in the sample matrix and their relative area responses were compared with that of the standard solution. Example chromatograms of fluoxetine spiked with 4-benzotrifluoride *t*-butyl ether, versus the standard solution and a fluoxetine blank are shown in Fig. 7. An evaluation of the area responses of the standard and spiked sample solutions indicated a spike recovery of 122.9%. The relative standard deviation (RSD) of the spike peak responses was 3.0%. This was considered to be very good recovery at the levels being evaluated. It was noted that peak widths of the spikes were slightly greater than that of the standard itself, possibly due to sample matrix effects to the column stationary phase upon injection. This was not regarded as significant.

Once method conditions were shown to be viable, a quantitative analysis was performed to determine levels of 4-benzotrifluoride *t*-butyl ether present in fluoxetine synthesized using potassium *t*-butoxide. Two different samples of fluoxetine (**I**) from the potassium *t*-butoxide process were assayed. The calibration curve described previously was used to establish values for 4-benzotrifluoride *t*-butyl ether (**II**) detected in each of the samples. Assay results indicated that 4-benzotrifluoride *t*-butyl ether (**II**) was present at levels of between 0.08 and 0.09% by weight (or 800 and 900 ppm) in the samples synthesized using the potassium *t*-butoxide route.

Assay precision was evaluated by quantifying, in triplicate, levels of 4-benzotrifluoride *t*-butyl ether (**II**) in a single sample of (**I**) generated by the potassium *t*-butoxide process. Concentrations of the replicates ranged between 24.0 and 26.1 mg/ml. The average quantity of ether detected in the sample solutions was determined to be 0.08% by weight versus the calibration curve described previously. The RSD for the three replicates was found to be 4.6%.

It was expected that the high loading of the analytical column using on-column injection, with relatively dirty samples, would ultimately have a detrimental effect. After a number of injections of concentrated ($\sim$20–25 mg/ml) fluoxetine free base samples, it was noted that the peak shape of the 4-benzotrifluoride *t*-butyl ether began to deteriorate, with the onset of tailing. However, the chromatography was immediately restored by simply replacing the retention gap with a new one. The improvement indicated that the tailing and peak broadening was due to material of low volatility trapped in the retention gap, and not on the analytical column itself. This was not a significant issue for the small study described here. In addition, the assay described here was developed to allow comparison of different synthetic processes using a relatively small number of samples, therefore, long-term assay ruggedness was not evaluated.

4. Conclusion

Using capillary GC/MS, with EI ionization, it has been confirmed that 4-benzotrifluoride *t*-butyl

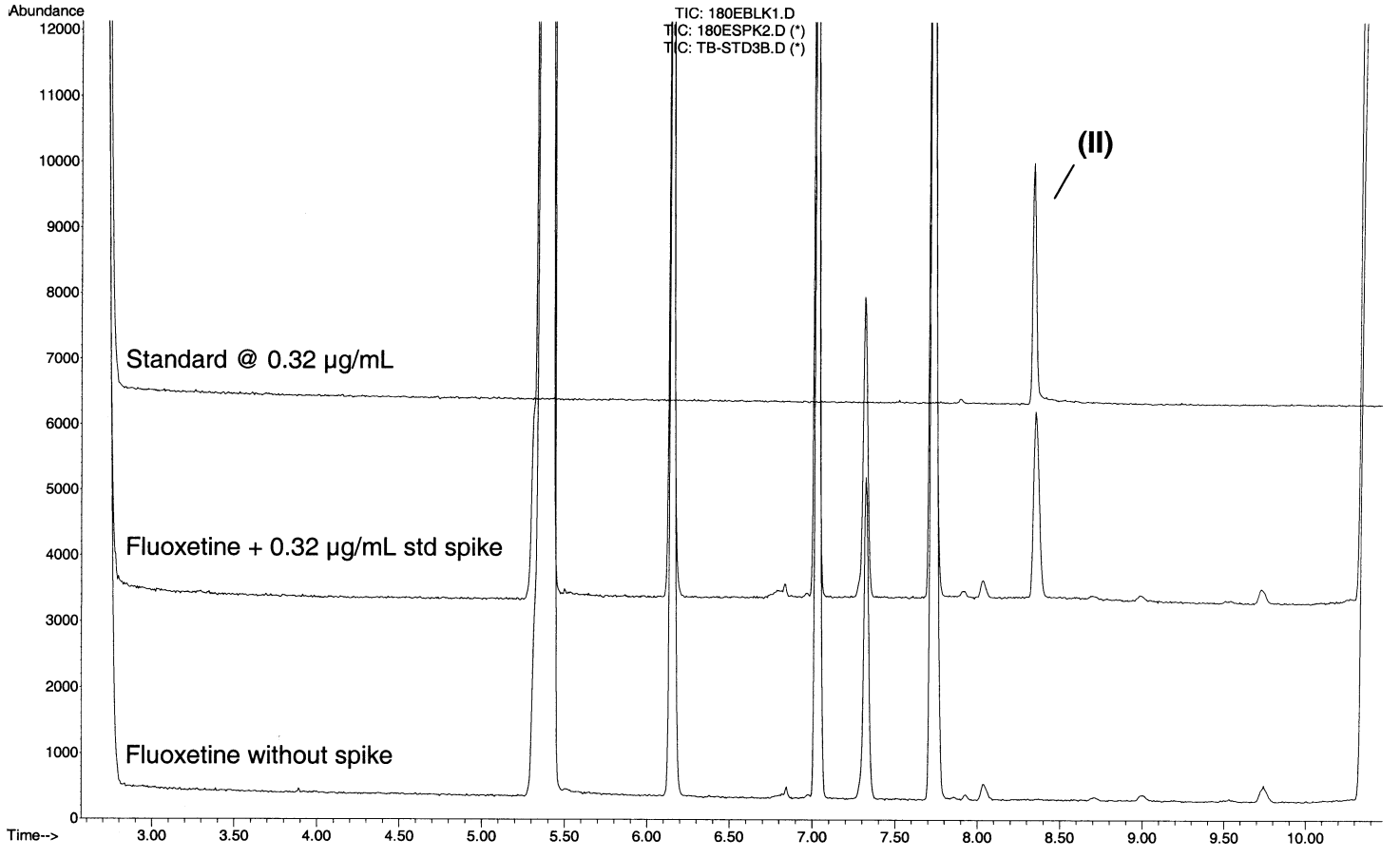

Fig. 7. Example of results from the spike recovery study, comparing spike versus non-spiked fluoxetine and the standard solution. In this case, the spike is equivalent to 12.9 ppm of the fluoxetine matrix.

ether is a reaction by-product existing in samples of fluoxetine (free base) synthesized using potasium *t*-butoxide as the base in the arylation step. In addition, chromatographic conditions have been developed which facilitate the use of SIM GC/MS for detection of 4-benzotrifluoride *t*-butyl ether at trace levels in free base samples of fluoxetine in the presence of 4-benzotrifluorophenol. The method provides sensitivity at the parts per million level, excellent linearity over a broad range, and adequate repeatability.

Acknowledgements

The authors would like to express thanks to C.B. Held and T.M. Koenig for their assistance with this effort.

References

[1] R.W. Fuller, D.T. Wong, D.W. Robertson, Med. Res. Rev. 11–1 (1991) 17–34.
[2] P. Benfield, R.C. Heel, S.P. Lewis, Drugs 32 (6) (1986) 481–508.
[3] P. Stark, C.D. Hardison, J. Clin. Psychiatry 46 (1985) 53–58.
[4] R.L. Zerbe, H. Rowe, G.G. Enas, D. Wong, N. Faried, L. Lemberger, J. Pharmacol. Exp. Ther. 232 (1985) 139–143.
[5] D.T. Wong, F.P. Bymaster, Res. Commun. Chem. Pathal. Pharmacol. 15 (1976) 221–231.
[6] B.B. Molloy, K.K. Schmiegel, US Patent 4,314,081, 1982.
[7] P.J. Kairisalo, P.J. Hukka, A.H. Jarvinen, US Patent 5,166,437, 1992.
[8] A. Kumar, H.D. Ner, S.Y. Dike, Tetrahedron Lett. 32 (1991) 1901–1904.
[9] Y. Gao, K.B. Sharpless, J. Org. Chem. 53 (1988) 4081–4084.
[10] M. Srebnik, P.V. Ramachandran, H.C. Brown, J. Org. Chem. 53 (1988) 2916–2920.
[11] E.J. Corey, G.A. Riechard, Tetrahedron Lett. 30 (1989) 5207–5210.
[12] A.T. Bottini, F.P. Corson, R. Fitzgerald, K.A. Frost, Tetrahedron 28 (18) (1972) 4883–4904.
[13] A.T. Bottini, F.P. Corson, R. Fitzgerald, K.A. Frost, Tetrahedron Lett. 54 (1970) 4753–4756.
[14] S.A. DiBlase, G.W. Gokel, J. Org. Chem 43 (1978) 447–452.
[15] R.H. Hales, J.S. Bradshaw, D.R. Pratt, J. Org. Chem. 36 (1971) 314–318.
[16] J.S. Bradshaw, R.H. Hales, J. Org. Chem. 36 (1971) 318–322.
[17] G.W. Gokel, D.J. Cram, C.L. Liotta, H.P. Harris, F.L. Cook, J. Org. Chem. 39 (1974) 2445–2446.
[18] L.A. Paquette, R.W. Houser, J. Org. Chem. 36 (1971) 1015–1016.
[19] D.E. Pearson, C.A. Buehler, Chem. Rev. 74 (1974) 45–86.
[20] D.D. Wirth, M.S. Miller, S.K. Boini, T.M. Koenig, Org. Process Res. Dev. 4 (6) (2000) 513–519.
[21] D.D. Wirth, B.A. Olsen, D.K. Hallenbeck, M.E. Lake, S.M. Gregg, F.M. Perry, Chromatographia 46 (1997) 511–513.
[22] 4-Benzotrifluoride *t*-butyl ether was prepared from the reaction of potassium *t*-butoxide and 4-chlorobenzotrifluoride or 4-fluorobenzotrifluoride. 1H NMR ($CDCl_3$, 300 MHz) d 7.52, (d, $J = 8.50$ Hz, 2 H), 7.04 (d, $J = 8.58$ Hz, 2 H), 1.38 (s, 9 H).