Evaluation of a Capillary Gas Chromatographic Impurity Test Procedure for 4-Hexylaniline

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Abstract

A capillary gas chromatographic test procedure for the detection and quantitation of impurities in the bulk intermediate, 4-hexylaniline, is evaluated and found to be accurate and precise. 4-Hexylaniline is dissolved in methanol and chromatographed isothermally at a temperature of 195°C on a 60-m × 0.32-mm 85% polyethylene glycol–15% dimethylsilicone blend (DX-4) film column. A flame ionization detector is used, and the impurities in the parent compound are estimated from peak areas on a percent basis compared with the area of the parent peak in the chromatogram. Response factors are determined for the known impurities. Validation of this test method includes a recovery study of known impurity spiked samples fortified in the range of 0.1–1% (w/w). A repeatability study is performed, consisting of the analysis of two different synthetic batch lots of 4-hexylaniline analyzed over three experimental run days using two chromatographic columns of different manufacturing lots. These data and other aspects of this test procedure are discussed.

Introduction

Testing for impurities in bulk intermediate compounds or final drug product within the pharmaceutical industry can be done by different chromatographic techniques. High-performance liquid chromatography (HPLC) is frequently used, which is demonstrated by the number of test methods published in the literature and in the current *United States Pharmacopoeia* (1–5). HPLC has a number of advantages over gas chromatographic (GC) testing; the avoidance of thermal degradation and the need for thermal volatility to perform GC analysis are the two main obvious advantages for the use of HPLC chromatographic testing. However, GC can be used effectively in impurity tests (6,7), and GC has a number of its own advantages. GC, when using the nearly universal flame ionization detector (FID), can be convenient and offer ease of use. The FID is more economical over mass spectrometric detectors, and the FID has the advantage of high sensitivity with a linear range of approximately seven orders of magnitude (8). The FID was originally introduced in 1958 by Ian McWilliam (9,10) and also has the known advantage of response factors with hydrocarbon and heterocyclic compounds generally approaching unity, (i.e., the response factors nearly equal one). Thus, GC analysis does have a "niche" in modern impurity testing of volatile and thermally stabile compounds. GC analysis was chosen for this work because of the previously mentioned reasons.

4-Hexylaniline is a synthetic intermediate for the production of *N*-(1,3-dithiolan-2-ylidene)-4-hexyl benzenamine (Figure 1), a compound under study for its pharmaceutical properties. *N*-(1,3-Dithiolan-2-ylidene)-4-hexyl benzeamine is in a class of compounds that demonstrate anti-inflammatory and related

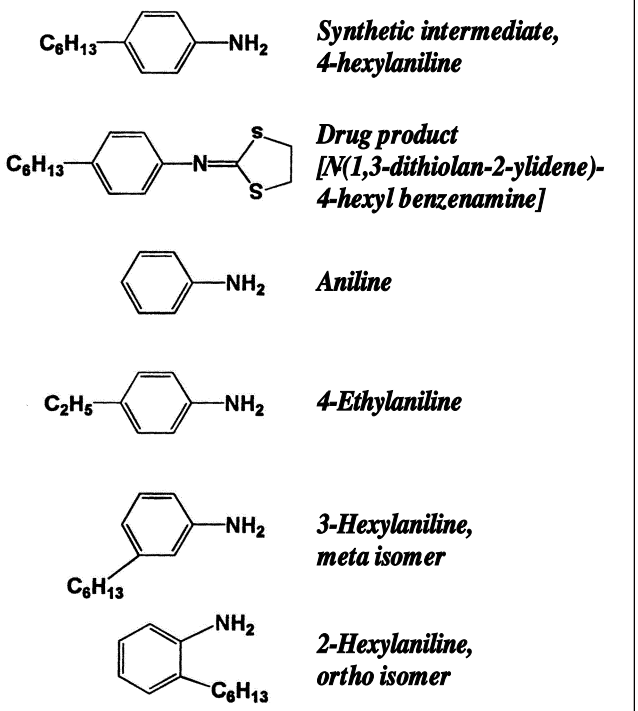

Figure 1. The chemical structures of the synthetic intermediate, 4-hexylaniline, drug product, *N*-(1,3-dithiolan-2-ylidene)-4-hexyl benzenamine, potential impurities of aniline, 4-ethylaniline, and positional isomers 3- and 2-hexylaniline.

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properties (11,12). It has also been reported that this class of non-steroidal anti-inflammatory compounds exhibit little activity on the central nervous system and have low gastrointestinal aggressiveness (11,12). Purity of the intermediate compound, 4-hexylaniline, is, therefore, vital for the production of the final bulk anti-inflammatory drug substance. Currently, there were no published validated methods to evaluate the purity of 4-hexylaniline and accurately determine the levels of known potential impurities; therefore, the development of a validated test method (13–16) was the major objective of this study. The potential impurities of 4-hexylaniline are shown in Figure 1. In the developed GC test procedure reported in this paper, a DX-4 column (85% polyethylene glycol–15% dimethylsilicone blend film) was used for the separation of the known potential impurities in 4-hexylaniline. A spiked recovery study of aniline, 4-ethylaniline, and 2- and 3-hexylaniline (the ortho and meta positional isomers) is used to verify the accuracy of the impurity test. Two actual synthetic batch lots of 4-hexylaniline were analyzed by this GC test procedure. These data, as well as many of the other facets of the development of this procedure, will be discussed.

Experimental

Reagents and chemicals

The methanol used for the sample dilution was HPLC grade (Burdick & Jackson, Muskegon, MI). GC purity was verified before use in dilution of any samples. The methanol was chromatographed using the GC conditions listed in this paper to verify that no impurity in solvent would interfere with the test of 4-hexylaniline. Aniline and 4-ethylaniline were commercially available (Sigma-Aldrich Chemicals, St. Louis, MO). All hexylaniline isomers were produced in-house.

Chromatographic conditions and apparatus

A Hewlett-Packard model 5890A (Agilent Technologies, Avondale, PA) capillary GC was used for all experiments. The chromatograph was equipped with a split injector, FID, and a DX-4 column (60-m × 0.32-mm i.d., 0.25-µm film thickness) (J&W Scientific, Agilent, Folsom, CA). The GC conditions consisted of operating the column and injection port at a temperature of 195°C, and detector temperature was 275°C. The carrier gas was helium at an approximate flow rate of 1.0 mL/min (constant head pressure of 17 psi). The detector make-up gas was nitrogen at a flow rate of 30 mL/min. The sample solution injection size was 1.0 µL with a split vent flow of 80 mL/min, which gave a split ratio of 80:1. The chromatographic run time was 40 min to allow time for all possible impurities to elute.

Sample preparation

Approximately 100 mg of 4-hexylaniline was accurately weighed into a 10-mL volumetric flask. The sample was dissolved in a few milliliters of methanol and then diluted to volume with methanol to make an approximately 10 mg/mL concentration of 4-hexylaniline solution.

Spiked solutions

Stock spiking solutions containing the other components were prepared in methanol. Spiked sample solutions containing 0.1%, 0.2%, 0.5%, and 1% (w/w) equivalent levels of aniline, 4-ethylaniline, 2-hexylaniline, and 3-hexylaniline were prepared for the spiked recovery study. Fresh solutions were prepared daily for the spiked recovery study. Bulk 4-hexylaniline batch lot A was used in all the impurity spiked solution prepared. A 1% (w/w) solution of 3-hexylaniline alone with the parent 4-hexylaniline compound was prepared for resolution testing of the two different DX-4 columns used in this study.

Calculations

The integrated peak areas were determined for each chromatogram. The area percent for all detected impurities were calculated as follows:

$$(A_i/SA) \times 100 = \% \text{ impurity} \qquad \text{Eq. 1}$$

where A_i is the area of the individual impurity or spike peak and SA is the summation of the area of all the peaks including the parent 4-hexylaniline (and excluding the initial peak from methanol solvent) in the chromatogram. Weight percent levels were calculated using determined response factors of the known impurities of 4-hexylaniline. Peak resolution between the parent 4-hexylaniline and 3-hexylaniline was calculated by the standard equation:

$$R_s = 2(t_{R2} - t_{R1})/(w_{b1} + w_{b2}) \qquad \text{Eq. 2}$$

where t_{R1} and t_{R2} are the retention times for the 4-hexylaniline and 3-hexylaniline peaks, respectively, and w_{b1} and w_{b2} are the peak widths at baseline for these peaks.

Results and Discussion

Column selection and chromatographic optimization

The 60-m DX-4 column was found to give the best separation of 3-hexylaniline from the parent 4-hexylaniline. The DX-4's high polarity and plate number (peak efficiency) made this separation possible. Various other columns were evaluated during this study, including those having film coatings of DB-5 (5% phenyl–95% methylpolysiloxane), DB-1701 (14% cyanopropyl–86%

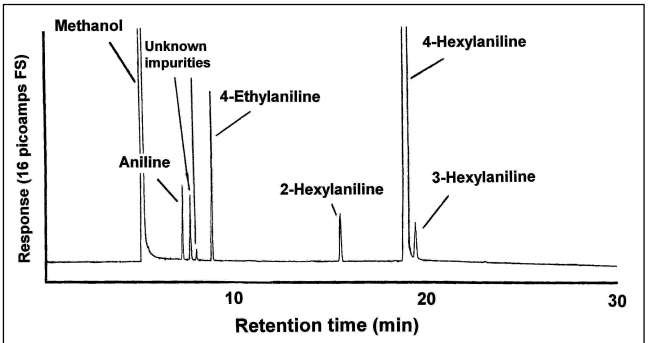

Figure 2. Chromatogram of a sample of 4-hexylaniline synthetic batch lot A with spikes of the known impurities. The sample was fortified at the 0.5% (w/w) level with aniline, 4-ethylaniline, 2-hexylaniline, and 3-hexylaniline.

methylpolysiloxane), and DB-225 (50% cyanopropylphenyl–50% methylpolysiloxane). All of these phases lacked selectivity and the ability to separate 3-hexylaniline from the parent 4-hexylaniline peak. The final optimized GC conditions using the DX-4 column described in this work gave baseline resolution of all the known impurities of 4-hexylaniline. 3-Hexylaniline, the meta isomer of the parent compound, was less well-resolved than the other known impurities. Although the meta isomer appeared near the tail of the 4-hexylaniline peak, it was reasonably separated from the parent peak for quantitation purposes; a chromatogram of a 0.5% (w/w) spiked solution is shown in Figure 2. The parent compound is basic in nature, adding to peak tailing problems in the separation of the meta isomer. Calculated resolution of meta isomer from the parent 4-hexylaniline was 1.5 for the lower performing DX-4 column using the 1% (w/w) spiked solution in this evaluation. The better performing DX-4 column gave a calculated resolution of 2.1 for the 1% (w/w) meta isomer spiked solution. Higher temperatures gave lower resolution for this critical separation, and temperatures lower than 195°C did not significantly improve resolution of the two isomers. Lower temperatures did show significant increases in retention times for the late eluting impurities found in the actual batches of 4-hexylaniline. Various temperature ramps that were evaluated did not improve this chromatographic system. Therefore, the 195°C column temperature was optimal for this impurity test procedure.

Table I. Recovery of Known Potential Impurities from Spiked 4-Hexylaniline Samples

(Synthetic batch lot A)			
Spike level (%, w/w)	Compound	Actual weight percent added	Recovery found by GC method* (%, w/w)
0	Aniline	0	0
	4-Ethylaniline	0	0.65†
	2-Hexylaniline	0	0
	3-Hexylaniline	0	0
0.1	Aniline	0.11	0.12
	4-Ethylaniline	0.10	0.73
	2-Hexylaniline	0.10	0.11
	3-Hexylaniline	0.10	0.10
0.2	Aniline	0.20	0.22
	4-Ethylaniline	0.19	0.85
	2-Hexylaniline	0.19	0.20
	3-Hexylaniline	0.19	0.18
0.5	Aniline	0.45	0.54
	4-Ethylaniline	0.49	1.18
	2-Hexylaniline	0.51	0.51
	3-Hexylaniline	0.49	0.48
1	Aniline	0.93	0.96
	4-Ethylaniline	0.93	1.53
	2-Hexylaniline	1.02	1.02
	3-Hexylaniline	0.93	1.04

* Recovery is based upon weight percent; response factors were used to calculate actual recovery.
† Synthetic batch lot A appeared to have a 0.65% level of 4-ethylaniline without any spike.

Recovery results and response factors

Recovery results for the known impurities using the lower resolving DX-4 column is shown in Table I; weight percent recoveries are reported for the known compounds, not area percent. Response factors were determined experimentally for all the known impurities; aniline and 4-ethylaniline had response factors of 0.8 and 0.9, respectively, though the positional isomers of 4-hexylaniline were determined to have the response factor of 1.0 using the FID. The 3-hexylaniline quantitation was very good, indicating that a resolution of 1.5 did not significant affect recovery accuracy. Batch lot A had some 4-ethylaniline impurity in it, as is shown in the zero spike level data displayed in Table I. Batch lot A appeared to have a 0.65% [area/area (A/A)] level of 4-ethylaniline as an impurity. The 2-hexylaniline and 3-hexylaniline position isomers were accurately determined in the spiked solutions. 3-Hexylaniline was determined to be 1.04% (w/w) for a 0.93% (w/w) spiked solution; aniline, 4-ethylaniline, and 2-hexyaniline had comparable recoveries as shown in Table I. The recovery study indicated that the test procedure was reasonably accurate for the estimation of known impurities of 4-hexylaniline.

Dilution solvent and sample stability

Methanol was used as the dilution solvent because of its short retention with the chromatographic conditions of the test procedure and because methanol has demonstrated stability when used in sample solutions of 4-hexylaniline. A 4-hexylaniline–methanol solution was chromatographed after both 1 h of standing and after standing overnight. No significant change in results of the impurities was noticed, indicating stability in the methanol sample matrix. Acetone was tried initially as a solvent, but an extra peak that increased with solution standing time was detected. This peak was attributed to the formation of a Schiff-base of 4-hexylaniline and acetone. Also, the high-grade methanol used to develop this procedure was found to have no significant impurities to interfere with the impurity testing of 4-hexylaniline.

Table II. Repeatability of the GC Test Procedure

Synthetic batch lot A						
Day	Column (DX-4)	Aniline	4-Ethyl aniline	2-Hexyl aniline	3-Hexyl aniline	Total impurities*
1	A	n.d.†	0.62	n.d.	n.d.	2.3
2	B	n.d.	0.64	n.d.	n.d.	2.2
3	B	n.d.	0.70	n.d.	n.d.	2.1
Mean		–	0.65	–	–	2.2
Synthetic batch lot B						
Day	Column (DX-4)	Aniline	4-Ethyl aniline	2-Hexyl aniline	3-Hexyl aniline	Total impurities*
1	A	0.08	0.34	n.d.†	5.0	8.1
2	B	0.10	0.34	n.d.	5.0	8.1
3	B	0.07	0.36	n.d.	5.0	8.1
Mean		0.08	0.35	–	5.0	8.1

* Unknown impurities are not listed individually in the Table, but they do form part of the total impurity levels on an area percent basis.
† n.d. = none detected.

Repeatability

The impurity level repeatability or method repeatability (13,14) from various manufacturing lots of the DX-4 GC column needs to be discussed. Two different synthetic batch lots of 4-hexylaniline were used over three separate test day periods. These samples were chromatographed using freshly prepared solutions and using the two different serial numbered DX-4 columns. The results of the analysis for each sample were similar for each day and each column as is shown in the results in Table II; results are reported in area percent (A/A%). 4-Hexylaniline batch lot A had a mean total impurity level of 2.2% (A/A) and batch lot B had mean total impurities of 8.1% (A/A). The total impurity level for sample batch lot A ranged from 2.1–2.3% (A/A); sample batch lot B had levels estimated at 8.1% each time for the 3 days of experimental analysis. The samples contained other unknown impurities; the known impurities were identified by spiking the sample solutions. Synthetic batch lot B contained a high level of 3-hexylaniline (5.0%), and this result was reproduced with both analytical DX-4 columns. The range of results for the known impurities in both samples was equally reproducible. The results in Table II were reported in two significant digits for the demonstration of the consistency of these impurity testing results. The retention times for both DX-4 columns were reproducible under the optimized conditions. Retention times for the parent 4-hexylaniline were within two minutes on both columns. Resolution performance of the meta isomer from the parent 4-hexylaniline has already been discussed in regards to the two DX-4 columns used.

Future work

The identification of the unknown impurities, as well as obtaining a more pure 4-hexylaniline, is beyond the scope of this manuscript and is part of planned future work. Different commercial sources of 4-hexylaniline are in the process of being evaluated by this procedure and considered for use in the synthesis of the *N*-(1,3-dithiolan-2-ylidene)-4-hexyl benzenamine drug product.

Conclusion

A test procedure to determine impurities in bulk 4-hexylaniline was evaluated and validated. Aniline, 4-ethylaniline, and 2- and 3-hexylaniline are resolved from the parent peak using a 60-m DX-4 (0.32-mm i.d.) and isothermal temperature setting at 195°C. An FID was used in this test procedure. A multilevel spiked recovery study ranging from 0.1% to 1% (w/w) demonstrated good accuracy for the known impurities found in 4-hexylaniline. Two synthetic batch lots of 4-hexylaniline were analyzed over a multiple-day trial period using two different DX-4 columns. Batch lot A had total impurities of approximately 2.2% (A/A), and batch lot B had total impurities of 8.1% (A/A). The test procedure appears to be capable of accurately estimating known potential impurities of bulk 4-hexylaniline.

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