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Short communication

Determination of chloroanilines in antibacterial soaps using cation-exchange chromatography with UV detection

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Abstract

A method is described for the rapid determination of 3,4-dichloroaniline and 4-chloroaniline in antibacterial soaps. Limits of detection (signal-to-noise ratio = 3) were 0.5 μg/g for 3,4-dichloroaniline and 2 μg/g for 4-chloroaniline. Including day-to-day and system-to-system variability, recoveries from a placebo spiked with 50 μg/g of each chloroaniline ($n = 20$) were 101.1 ± 1.6% for 3,4-dichloroaniline and 102.9 ± 7.6% for 4-chloroaniline. The method uses UV detection and is applicable to multiple formulations.

1. Introduction

Triclocarban [chemical name: N-(4-chlorophenyl)-N′-(3,4-dichlorophenyl)urea] has shown excellent bacteriostatic activity [1] and is used in this capacity in antibacterial soaps. Since both 3,4-dichloroaniline and 4-chloroaniline are possible degradation products of Triclocarban (TCC), it is important to monitor these compounds in finished soap products.

To date numerous procedures have been published for the determination of various chloroanilines [2–7], using primarily reversed-phase HPLC. In soap, such an approach is complicated by the presence of fragrance components which elute in the vicinity of the chloroanilines. Since these components vary from formulation to formulation, it is difficult to develop a single method applicable to multiple products. Additionally, as a result of the dissimilarity in the polarity of the chloroanilines and TCC, gradient elution is necessary. Both of these problems are obstacles in developing a single method for quality control laboratories which is both rugged and fast.

Several authors [4–7] have used acidic conditions and cation exchange as a means of isolating chloroanilines. This approach is taken here, not as a means of isolation, but as a means of separation. The use of cation-exchange chromatography has eliminated the need for gradients and interferences from matrix components

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for numerous soaps investigated. The method as described uses UV detection and allows for analysis in less than 15 min.

2. Experimental

2.1. Chromatography

Analyses were performed using either of two systems: (1) a Shimadzu LC 600 isocratic pump (Shimadzu Corporation, Kyoto, Japan) with a Shimadzu SPD-6A UV detector and a Waters 712 WISP autoinjector (Waters, Milford, MA, USA); or (2) a Waters 590 pump with a Kratos Spectroflow 783 programmable absorbance detector (Applied Biosystems, Foster City, CA, USA) and a Waters 710 WISP autoinjector. The separations were achieved on 150 × 4.6 mm Zorbax 300 SCX columns (Mac-Mod Analytical, Chadds Ford, PA, USA) using a mobile phase consisting of 10 m*M* potassium dihydrogen phosphate in acetonitrile–water (30:70, v/v) adjusted to pH 2.5 with phosphoric acid (15 Megaohm cm water was obtained from a Milli-Q reagent water system from Millipore Corporation, Milford, MA; all other mobile phase components were purchased from J.T. Baker, Phillipsburg, NJ, USA). The mobile phase flow-rate was 1.5 ml/min, the injection volume was 200 μl and UV detection was at 240 nm. Data were collected using PE Nelson Access Chrom. Software (Perkin-Elmer, Norwalk, CT, USA).

2.2. Sample preparation

Soap samples and placebos (without TCC) were prepared in-house. The soap samples were finely grated and homogeneous 4-g portions were stirred with 100 ml of acetonitrile. Working sample solutions were obtained by diluting the resulting slurries 10:50 with acetonitrile–water (30:70, v/v) and filtering through 0.45-μm filters (VWR Scientific, South Plainfield, NJ, USA). Standards of 3,4-dichloroaniline and 4-chloroaniline (Aldrich, Milwaukee, WI, USA) were prepared fresh daily. Stock standards containing 50–110 mg/100 ml of each compound in acetonitrile were serially diluted to working concentrations using acetonitrile–water (30:70, v/v).

For validation work, soap placebos were spiked with 5 mg/g TCC (recrystallized in hexane) and the appropriate amount of each chloroaniline. Additional sample preparation steps were as for the samples.

3. Results and discussion

The use of reversed-phase HPLC with UV detection for the analysis of chloroanilines in soaps is not rugged enough to be used for multiple formulations, principally due to interference from fragrance components which vary greatly from brand to brand. Greater specificity may be obtained by using electrochemical detection [4,7], but some fragrance components are also susceptible to oxidation.

Both 4-chloroaniline and 3,4-dichloroaniline are ionized at a pH of 2.5 and as a result can potentially be analyzed as cations. However, since soap matrices contain nonpolar materials, it is important that the stationary phase shows little reversed-phase behavior or alternatively, is compatible with organic solvents. Of several cation-exchange materials evaluated, Zorbax SCX 300 met these criteria most suitably and, as shown in Fig. 1, provided good retention behavior and acceptable peak shapes for both chloroanilines (peak asymmetry factors were less than 1.3 for both species). Most notably, the chromatogram shows little retention of any significant components other than the chloroanilines, which suggests good method specificity.

Retention is dramatically affected by both potassium dihydrogen phosphate and acetonitrile concentrations. In developing the outlined method, acetonitrile concentrations were varied from 20 to 60% (v/v); and potassium dihydrogen phosphate concentrations from 5 to 25 m*M*. Increasing the acetonitrile concentration reduced the retention of both species, but the reduction was more pronounced for 3,4-dichloroaniline. Increasing the concentration of potassium

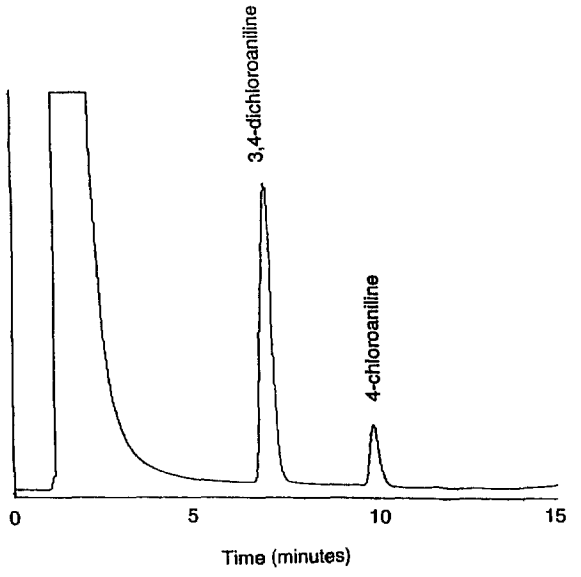

Fig. 1. Chromatogram of 3,4-dichloroaniline and 4-chloroaniline in a commercially available soap. Column: Zorbax 300 SCX (150 × 4.6 mm); mobile phase: 10 m*M* potassium dihydrogen phosphate in acetonitrile–water (30:70, v/v) adjusted to a pH of 2.5 with phosphoric acid; flow-rate: 1.5 ml/min; detection: UV at 240 nm; injection volume: 200 μl.

dihydrogen phosphate reduced the retention of 4-chloroaniline to a greater extent. As a result of these effects, large differences in selectivity (including changes in elution order) were observed between the systems investigated.

From a ruggedness perspective, it is desirable to use a mobile phase for which minor changes in composition do not dramatically change the chromatography. The selected mobile phase of 10 m*M* potassium dihydrogen phosphate in acetonitrile–water (30:70, v/v) adjusted to pH 2.5 with phosphoric acid, was chosen on the basis of not only providing adequate resolution, but also representing a composition where minor changes in the concentrations of acetonitrile and potassium dihydrogen phosphate do not change the chromatography significantly. An injection volume of 200 μl was used to provide suitable concentration detection limits.

The conditions outlined were used to determine method linearity for 4-chloroaniline and 3,4-dichloroaniline levels ranging from 200 to 600 ng/ml. These levels correspond to chloroaniline in soap levels of 25–75 μg/g based on the sample preparation procedure described. For both compounds, linearity was evaluated based on a 15-point calibration curve. F-tests for regression and lack of fit showed both curves to be linear at the 95% confidence level. Limits of detection, at 3 times the signal-to-noise level, were determined to be 4 ng/ml for 3,4-dichloroaniline and 16 ng/ml for 4-chloroaniline, which indicates that it should be possible to quantitate (LOQ = 10 × noise) each chemical species in soap at concentrations of 7 μg/g or less.

To evaluate the quantitative capabilities of the method, spiked placebos were prepared in triplicate to contain 25, 50 and 75 μg/g of each chloroaniline and the recoveries were determined (Table 1). Overall recoveries for the spiked placebos were 103.0 ± 2.8% for 3,4-dichloroaniline and 108.4 ± 6.8% for 4-chloroaniline.

Table 1
Recoveries of 4-chloroaniline and 3,4-dichloroaniline from a spiked soap placebo

Amount of each chloroaniline in soap (ppm)	Recovery (%)[a]	
	4-Chloroaniline	3,4-Dichloroaniline
25	115.6 ± 4.7	106.5 ± 0.5
50	107.6 ± 4.4	102.2 ± 0.8
75	102.0 ± 1.9	100.4 ± 0.3
Overall	108.4 ± 6.8	103.0 ± 2.8

$n = 3$ for each level.
[a] Average ± standard deviation.

Additionally, a single preparation was made containing 50 μg/g of each chloroaniline and was analyzed five times by each of two operators on each of two days (for a total of 20 determinations). Each operator used a separate HPLC system and new calibration curves were prepared for each of the four data sets. The results, summarized in Table 2, show overall recoveries of 101.1 ± 1.6% for 3,4-dichloroaniline and 102.9 ± 7.6% for 4-chloroaniline.

To validate the methodology for other soap formulations, placebos of the soaps were analyzed and the resulting chromatograms were evaluated for interferences as the basis for method suitability. (It was assumed that other soap matrices are similar enough that the matrix tested adequately models the release of chloroanilines from the matrix). For four placebos tested to date, no significant interferences have been observed. Additional evaluation is required to validate the transfer of the method to quality control sites and the applicability of the method must strictly be tested on a formulation-to-formulation basis. However, the specificity of the method for the formulations tested to date is encouraging.

References

[1] D.J. Beaver, D.P. Roman and P.J. Stoffel, J. Am. Chem. Soc., 79 (1957) 1236.
[2] A.H.M.T. Scholten, U.A.Th. Brinkman and R.W. Frei, J. Chromatogr., 218 (1981) 3.
[3] K. Thyssen, J. Chromatogr., 319 (1985) 99.
[4] M.W.F. Nielen, R.W. Frei and U.A.Th. Brinkman, J. Chromatogr., 317 (1984) 557.
[5] R.B. Geerdink, J. Chromatogr., 445 (1988) 273.
[6] A. Di Corcia and R. Samperi, Anal. Chem., 62 (1990) 1490.
[7] V. Coquart and M.-C. Hennion, Chromatographia, 37 (1993) 392.

Table 2
Reproducibility of 20 replicate determinations of a single preparation containing 50 ppm of each chloroaniline

Recovery[a]			
Operator 1		Operator 2	
Day 1	Day 2	Day 1	Day 2
4-Chloroaniline			
107.3 ± 0.5	112.1 ± 2.5	97.3 ± 2.9	94.7 ± 3.1
Overall 102.9 ± 7.6			
3,4-Dichloroaniline			
102.0 ± 1.1	100.4 ± 0.5	102.7 ± 1.1	99.3 ± 0.8
Overall 101.1 ± 1.6			

[a] Numbers are expressed as average percent recoveries ± the standard deviation; each operator/day data set is based on 5 determinations.