Identification and Quantitation of DTPA and Other Aminocarboxylate Chelating Agents in Bar Soaps by HPLC

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A high pressure liquid chromatography method is described for the identification and quantitation of aminocarboxylate chelating agents in bar soaps. The method involves reacting the sample with cupric sulfate which precipitates the soap and forms a water soluble aminocarboxylate-copper complex. The complex is identified and quantified versus suitable standards by HPLC using an anion exchange column with dilute sulfuric acid mobile phase and UV measurement at 254 nm. The method is specific and sensitive with a detection limit of approximately 0.005%. Average recoveries of about 85% were obtained for soap samples spiked with individual chelating agents in the range of 0.01% to 0.10%.

Low levels of aminocarboxylate chelating agents frequently are incorporated into bar soaps to complex traces of metallic ions whose presence can lead to autoxidation, rancidity and other undesirable effects (1). It is, therefore, important to have analytical methodology to monitor the presence and concentration of these reagents.

Although numerous methods have been reported for the determination of aminocarboxylate chelating agents, especially EDTA (the disodium salt of ethylenediaminetetraacetic acid) using a variety of techniques including titrimetry (2), colorimetry (3), gas chromatography (4) and high pressure liquid chromatography (HPLC) in different matrices such as foodstuffs (5), boiler water (6) and ophthalmic preparations (7), no methods were found dealing specifically with soap products.

The HPLC method of Parkes (8), which involved chelating agent analysis in the form of its copper complex, used a reverse phase paired ion approach and reported good results for EDTA and HOEDTA (the trisodium salt of N-hydroxyethylenediaminetriacetic acid) but stated that the copper complex of DTPA (the pentasodium salt of diethylenetriaminepentaacetic acid) did not elute from the column.

This paper describes the use of an anion exchange HPLC column for copper chelant analysis which allows DTPA as well as EDTA and HOEDTA to be determined in soap products.

EXPERIMENTAL

Apparatus. Liquid chromatographic analysis was performed with a Hewlett-Packard Model 1084B liquid chromatograph equipped with a Hewlett Packard 79850B LC terminal, variable volume autosampler, variable wavelength UV-visible detector, and a Wescan anion R analytical column (250 ÷ 4 mm) and guard cartridge (Wescan Instruments, Santa Clara, California).

Reagents and chemicals. the chelating agents were commercial grade EDTA (i.e., Versene NA, 99% active $Na_2H_2EDTA.2H_2O$), DTPA (i.e., Versenex 80, 40% active Na_5DTPA), and HOEDTA (i.e., Versenol 120, 41% active $Na_3HOEDTA$) purchased from Dow Chemical Co. Water was obtained from a Milli-Q (Millipore) water purification system. Cupric sulfate solution, 0.05 M, was prepared from reagent grade cupric sulfate pentahydrate. The mobile phase, 0.003 M sulfuric acid, was prepared by diluting high purity sulfuric acid (i.e., J.T. Baker Ultrex) with Milli-Q purified water. This solution was then filtered through a 0.5-μm membrane filter and degassed under vacuum.

Preparation of standards and samples. Individual standard chelating agent stock solutions (i.e., DTPA, EDTA and HOEDTA) of 400 mg/l (active level) were prepared and then quantitatively diluted with distilled water to prepare 40 mg/l standard working solutions. Standard copper chelant solutions were prepared by mixing 4.0-ml aliquots of standard working solutions with 6.0 ml of 0.05 M copper sulfate. A portion of each mixture was filtered through disposable 0.5-μm filter units into septum sample vials and capped securely. Calibration curves were prepared by further dilution of the standard working solutions (prior to cupric ion reaction) and showed linear response over a wide concentration range as determined by peak height measurements.

Sample preparation involved weighing four g (to the nearest 0.01 g) of finely divided soap product into a 250-ml Erlenmeyer flask, adding 100 ml of distilled water, covering with a watch glass and dissolving the sample with gentle heating and magnetic stirring. The soap solution was allowed to cool to room temperature, and a 4.0-ml aliquot removed promptly (to avoid gelation) and reacted in a 20-ml screw cap scintillation vial with 6.0 ml of 0.05 M copper sulfate. The mixture was shaken thoroughly and the precipitate allowed to settle for a few minutes. A portion of the supernatant was filtered through a disposable 0.5-μm filter unit into a septum vial and capped.

TABLE 1

Recoveries of DTPA from Spiked Placebo pellets[a]

DTPA added (%)	Recovery (%)
.054	83
.054	87
.054	86
.054	86
.054	86
.097	84
.097	85
.097	90
Average ± standard deviation	85.9 ± 2.1%

[a]Spiking was done by adding aliquots of a standard solution of DTPA to samples in flasks during the dissolution step.

The copper chelate solutions are quite stable and can be safely stored at room temperature for several days.

Chromatographic conditions. Flow rate was 1.5 ml/min, injection volume was 50 μl, detector wavelength was 254 nm, sensitivity setting was 0.006 aufs (absorbance units full scale). Under these conditions, the copper chelate retention times were 3.5 min for HOEDTA, 6.2 min for DTPA, and 6.9 min for EDTA.

RESULTS AND DISCUSSION

The primary purpose of this work was to develop a specific and sensitive method for determining DTPA in commercial bar soaps. Because the copper chelate approach using HPLC with UV detection apparently was effective for EDTA and HOEDTA, but not DTPA, using a reverse phase paired-ion system, another type of HPLC column and mobile phase was investigated.

The Wescan resin-based anion exchange column was found to provide an effective DTPA-copper chelate peak using 0.005 M sulfuric acid mobile phase and UV detection at 254 nm. Further studies also demonstrated reproducible and specific copper chelate peaks for EDTA and HOEDTA under these same conditions.

In addition to providing effective copper chelate water soluble reaction products, cupric ion treatment of soap solutions is also beneficial in that it acts as a precipitating agent for soap fatty acids, which can then be removed by filtration. Thus, the copper reaction provides a useful sample preparation and clean-up step for soap products.

DTPA methodology validation studies were conducted in which placebo soap pellets (containing all ingredients but DTPA) were intentionally fortified with known levels of DTPA. The samples, which showed no peaks in the DTPA retention region, were spiked

TABLE 2

Reproducibility of Method for DTPA in Bar Soap

Bar A (prepared with 0.10%) DTPA found (%)	Bar B (prepared with 0.05%) DTPA found (%)
0.097	0.045
0.107	0.046
0.101	0.047
0.103	0.049
0.106	0.049
Average ± standard deviation: 0.103 ± 0.004	0.047 ± 0.002
Percent relative standard deviation: 3.9%	3.8%

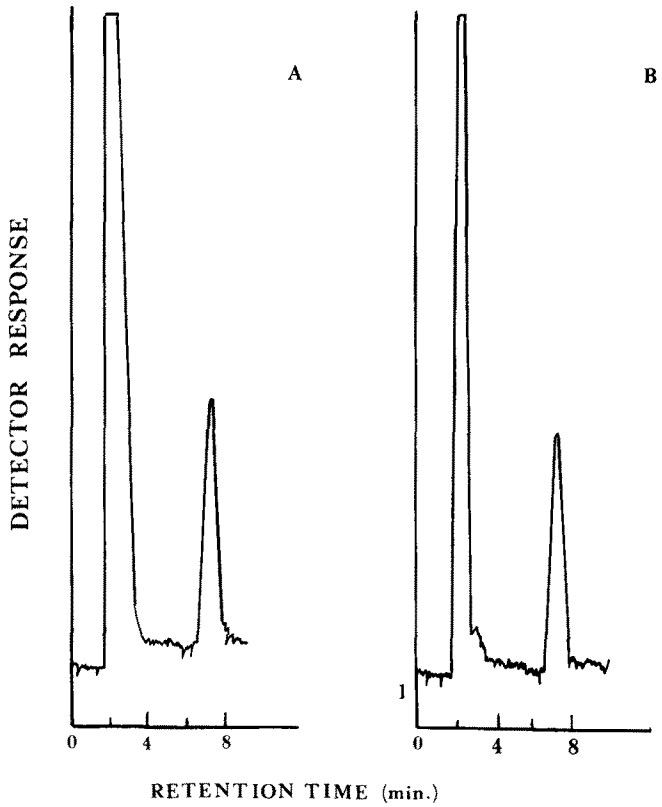

FIG. 1. HPLC trace of: A, DTPA standard (400 ng injected, equivalent to 0.05% in product); B, soap sample containing 0.05% DTPA.

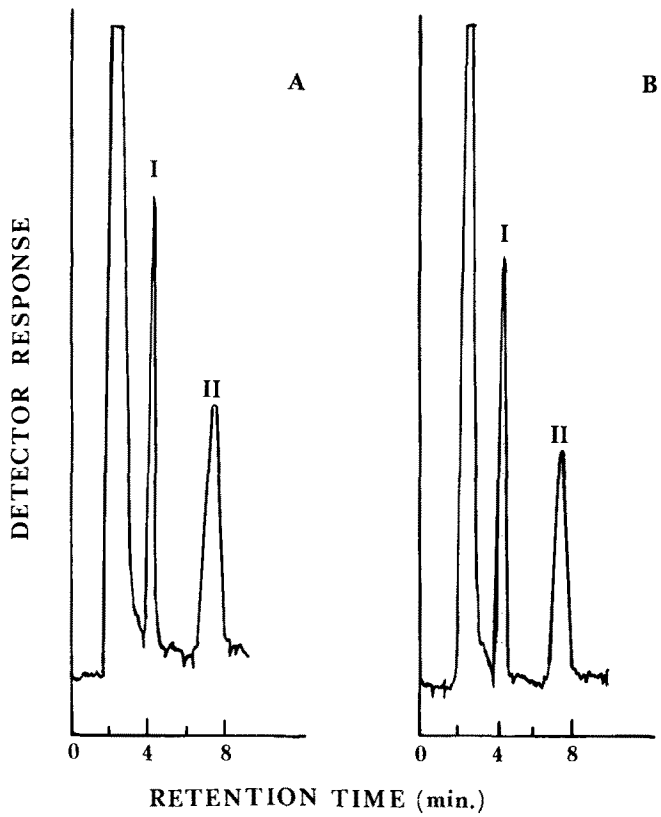

FIG. 2. HPLC trace of: A, Standard mixture of HOEDTA (I) and DTPA (II); concentrations equivalent to 0.02 % (I) and 0.05% (II) in product; B, soap sample prepared with 0.05% DTPA and spiked with 0.02 % HOEDTA.

by adding known amounts of DTPA standard stock solution to four g of soap pellets prior to mixing with a total of 100 ml of distilled water. The recovery results, which averaged 85.9%, are summarized in Table 1. Typical HPLC traces for a DTPA standard and a DTPA-containing soap sample are shown in Figure 1.

Soap samples spiked with EDTA showed average recoveries of 80% at the 0.03% level, whereas HOEDTA showed 90% recoveries at the 0.01% spike level.

Additional DTPA accuracy and precision data were obtained by running quintuplicate determinations on fresh production samples of two commercial bar soaps having somewhat different compositions. Bar A initially prepared with 0.10% DTPA showed analysis results of 0.103 0.004%, whereas bar B, formulated with 0.05%, showed 0.0472 0.002% DTPA (Table 2).

Although external standards were used to calculate the data summarized in Table 2, it has been demonstrated in our laboratory that HOEDTA can be used satisfactorily as an internal standard for DTPA (or EDTA) assays. An HPLC trace of a standard DTPA-HOEDTA mixture along with a DTPA soap sample spiked with HOEDTA are shown in Figure 2.

As indicated by the retention values (experimental section), DTPA and EDTA are not completely resolved from each other. Thus, it would be difficult to quantitate a mixture of these two materials if they happened to be used together in the same product. Each material, if used separately, however, can be readily identified and quantified.

REFERENCES

1. Milwidsky, B., *HAPPI 21*(11):87 (1984).
2. Ross, J., and M. Frant, *Anal. Chem. 41*:1900 (1969).
3. *Keys to Chelation*, Dow Chemical Co., Midland, MI, 1969, pp. 1-20.
4. Blank, M., and F. Snyder, *J. Chromatogr. 170*:379 (1979).
5. Perfetti, G., and C. Warner, *J. Assoc. Off. Anal. Chem. 62*:1092 (1979).
6. Venezky, D., and W. Rudzinski, *Anal. Chem. 56*:315 (1984).
7. Bauer, J., D. Heathcote and S. Krogh, *J. Chromatogr. 369*:422 (1986).
8. Parkes, D., M. Caruso and J. Spradling, *Anal. Chem. 53*:2154 (1981).

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