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Note

Analysis of cetylpyridinium chloride in the polysaccharide, chondroitin sulfate, via high-performance liquid chromatography

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A rapid, specific procedure was sought for the analysis of cetylpyridinium chloride (I) at trace contaminant levels. Several methods have been reported by others in the literature. Chatten and Okamura[1] described the analysis of many quaternary ammonium compounds using acid-dye techniques. Moore and Stretton[2] described their use of high-voltage agar gel electrophoresis for detecting preservatives in pharmaceutical and cosmetic products. Others reported determining organic ammonium compounds using titration methods[3–6]. The USP takes this approach with the use of bromphenol blue and its subsequent titration with tetraphenylboron[7]. The criteria for a rapid and specific method at the levels of (I) to be investigated were not fulfilled in any of the reports in the literature.

Recent papers by the authors' group[8,9] showed the applicability of high-performance liquid chromatography (HPLC) for the determination of a similar quaternary ammonium compound, benzalkonium chloride, at the desired levels. Modification of those procedures produced the work reported here.

EXPERIMENTAL

Apparatus

An HPLC instrument (Beckman Instruments, Fullerton, CA, U.S.A.) equipped with a 254-nm detector (Model 160), loop injector, and pump was used for the chromatographic work. Peak areas were obtained using a Hewlett-Packard electronic integrator (Model 21MX Computer Series, Avondale, PA, U.S.A.). The reversed-phase column was a microparticulate cyano, μBondapak CN (Waters Assoc., Milford, MA, U.S.A.), 30 cm × 3.9 mm.

Chemicals

The ion-pairing reagent, tetramethylammonium hydroxide pentahydrate, was used as supplied (MCB, Norwood, OH, U.S.A.). Methanol and acetic acid were of reagent grade quality. Water was distilled and deionized. Before use, the mobile solvent was filtered through a 1.2-μm filter (Whatman, Clifton, NJ, U.S.A.). The standard (I) was analyzed using the USP procedure at greater than 98% purity (Fine Organics, Lodi, NJ, U.S.A.). Samples of chondroitin sulfate were from bovine and shark sources.

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Methodology

The mobile phase was made in two steps. The ion-pairing reagent was prepared by dissolving 3.6 g tetramethylammonium hydroxide pentahydrate in about 950 ml of water. The pH was adjusted to 3.5 with acetic acid and the solution was made to 1000 ml. To 400 ml of the above solution, 600 ml of methanol were added, and the solution was mixed and filtered. A stock standard of (I) at 0.10% was prepared in water. Working standards of 1 and 10 ppm were prepared by diluting the appropriate aliquot of stock solution with the mobile phase.

Samples of chondroitin sulfate were prepared by accurately weighing 40 mg and placing it in 10-ml volumetric flasks. About 9 ml of mobile phase were added and sonication was used to dissolve each completely. Mobile phase was then added to bring to mark, and all were mixed well. The HPLC system was started using conditions summarized in Table I and a stable baseline was obtained.

Several standards of (I) were run to show injection-to-injection reproducibility. Samples of chondroitin sulfate were then run to determine proper levels of (I) to investigate. Appropriate loop and standard changes were made and a complete run was started, with standards injected after every third sample.

RESULTS AND DISCUSSION

Cetylpyridinium chloride is used as a precipitating agent in the purification of chondroitin sulfate. A series of saline–methanolic washes are then carried out to remove (I). Because (I) would be considered a contaminant of chondroitin sulfate, its removal must be assured. The development of this procedure became necessary before further research could be conducted with chondroitin sulfate.

Several different ion-pairing reagents were tested in the development of this analytical procedure. The acetate and propionate buffer systems used by the author and co-workers in their published works on benzalkonium chloride produced peaks of acceptable retention. However, spiking samples of chondroitin sulfate with (I) resulted in recoveries of only 50%. Other systems, such as ion-pairing with 1-pentane- or 1-hexanesulfonic acid, or buffering with ammonium phosphate, produced similar results. The use of 0.02 *M* tetramethylammonium hydroxide gave the desired results of a chromatographically retained peak and close to 100% recoveries from spiked chondroitin sulfate. In this case, use of this ion-pairing agent appears to affect the

TABLE I

HPLC CONDITIONS FOR THE DETERMINATION OF CETYLPYRIDINIUM CHLORIDE IN CHONDROITIN SULFATE

Column	μBondapak cyano (Waters Assoc., reversed-phase mode)
Mobile phase	Methanol–tetramethylammonium hydroxide pentahydrate (0.02 *M*) (60:40) adjusted to pH 3.5 with acetic acid
Flow-rate	2.0 ml/min
Detector	0.01 a.u.f.s.
Injection loops	30 μl for 10 ppm (I), or 250 μl for 1 ppm (I)
Chart speed	0.25 cm/min
Analysis time	8 min between injections

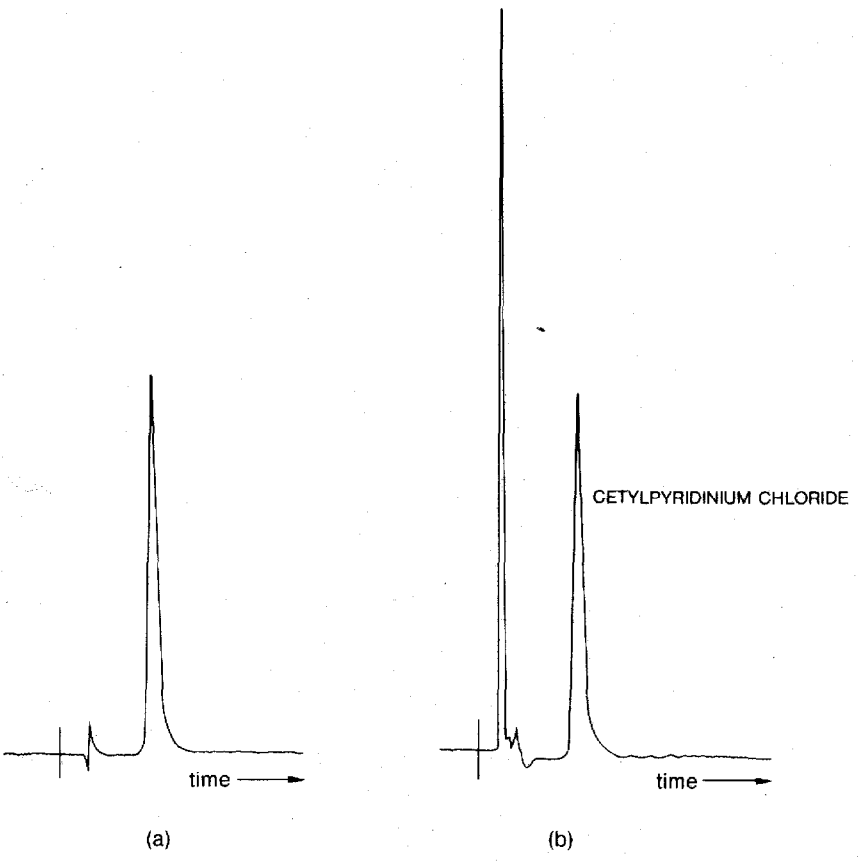

Fig. 1. (a) Cetylpyridinium chloride standard at 10 ppm. (b) same standard spiked into bovine chondroitin sulfate (4 mg/ml).

TABLE II

STANDARD CETYLPYRIDINIUM CHLORIDE REPRODUCIBILITY (VIA AREA INTEGRATION)

	10 ppm Std	*1 ppm Std*
	40865	49784
	40520	51513
	39008	49797
	38871	48360
	40050	51009
		50232
		49391
		52753
Mean	39863	50355
Relative standard deviation	±2.2%	±2.7%

TABLE III

STANDARD CETYLPYRIDINIUM CHLORIDE (10 ppm) IN THE PRESENCE OF CHONDROITIN SULFATE (4 mg/ml)

Presence of chondroitin sulfate indicated by CS.

Ingredients	*Peak height*	*Recovery (%)*
Std	829	
Std + CS	806	97.6
Std	822	
Std + CS	808	98.4
Std	820	
Std + CS	811	98.2
Std	831	
Std + CS	822	98.9
Std	831	
Std + CS	820	98.7
Mean		98.4%
Relative standard deviation		±0.5%

interaction of (I) with the chondroitin sulfate, making (I) accessible for chromatographic assay.

Fig. 1a is a typical chromatogram of a cetylpyridinium chloride standard at 10 ppm. Fig. 1b is the same standard spiked into bovine chondroitin sulfate (4 mg/ml).

Table II presents the reproducibility of a standard at both 1 and 10 ppm (no chondroitin sulfate present).

Tables III and IV present recovery data of spiked amounts of cetylpyridinium chloride standard in 4 mg/ml chondroitin sulfate (previously shown to be free from cetylpyridinium chloride). Table IV also shows equivalence of measuring either peak area or peak height. Thus, at both 0.025% w/w and 0.25% w/w levels, recoveries of (I) are equivalent and close to 100%. It appears that any effect of chondroitin sulfate

TABLE IV

STANDARD CETYLPYRIDINIUM CHLORIDE (1 ppm) IN THE PRESENCE OF CHONDROITIN SULFATE (4 mg/ml)

Ingredients	*Area*	*Recovery (%)*	*Peak height*	*Recovery (%)*
Std	54549		419	
Std + CS	52488	100.2	398	96.7
Std	50273		402	
Std + CS	47524	96.1	393	98.0
Std + CS	48928	98.9	400	99.8
Std	48669		400	
Std + CS	48660	97.0	404	97.5
Std	51325		429	
Mean		98.1		98.0
Relative standard deviation		±1.6%		± 1.3%

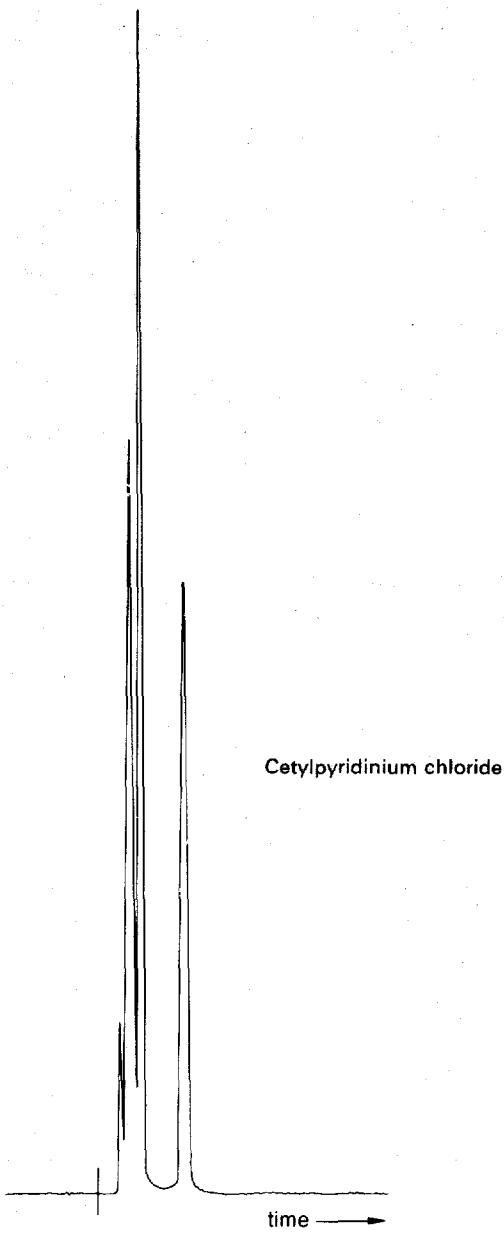

Fig. 2. Cetylpyridinium chloride in a commercially available mouthwash.

on the analysis of cetylpyridinium chloride is minimal. The procedure does make the assumption that any cetylpyridinium chloride present in chondroitin sulfate is released upon dissolution in the mobile phase and subsequent chromatography.

A calibration curve was determined for levels of (I) between 0.25 ppm and 15 ppm. The graph was linear and passed very close to zero ($r = 0.9998$). Using the

larger injection loop for sampling, about 0.05 ppm would be the lower limit for detection of (I). This corresponds to approximately 0.00125% (I) in chondroitin sulfate.

Due to the ion-pairing reagent used, low plate counts for (I) are normally encountered. Typical values are in the range of $n = 300$–400 plates for the column specified. Values less than $n = 100$ would not be acceptable.

Although the analysis procedure was developed to determine the presence of (I) as a trace contaminant in a solid (chondroitin sulfate), it may also be useful to others as a routine method to determine (I) in marketed pharmaceuticals. To demonstrate this, one bottle of a commercially available mouthwash was assayed for its cetylpyridinium chloride content. Although it had been held at ambient temperature for 54 months and was 9 months past expiration date, the assayed concentrations of (I) were 0.0459%, 0.0471% and 0.0458%. The *Physicians' Desk Reference for Nonprescription Drugs*[10] lists the concentration of (I) in the product to be 0.05% (1/2000). Fig. 2 is the chromatogram that was obtained.

NOTE

The HPLC mobile phase has a very deleterious effect on the column by eventually causing bed slumping. If continuous use of the column is expected, investigation into the possible use of a cyano guard column is advised.

REFERENCES

1 L. G. Chatten and K. O. Okamura, *J. Pharm. Sci.*, 62 (1973) 1328.
2 K. E. Moore and R. J. Stretton, *J. Chromatogr.*, 156 (1978) 211.
3 E. D. Carkhuff and W. F. Boyd, *J. Amer. Pharm. Ass. Sci. Ed.*, 43 (1954) 240.
4 D. M. Patel and R. A. Anderson, *Drug Stand.*, 26 (1958) 189.
5 J. T. Cross, *Analyst (London)*, 90 (1965) 315.
6 S. Pinzauti and E. LaPorta, *Analyst (London)*, 102 (1977) 938.
7 *The United States Pharmacopeia, 20th rev.*, Mack Printing Company, Easton, PA, 1980, p. 127.
8 R. C. Meyer, *J. Pharm. Sci.*, 69 (1980) 1148.
9 D. Marsh and L. Takahashi, *J. Pharm. Sci.*, 72 (1983) 521.
10 B. B. Huff (Editor), *Physicians' Desk Reference for Nonprescription Drugs*, Charles E. Baker, Jr., Medical Economics Company, Inc., Oradell, NJ, 3rd ed., 1982, p. 589.