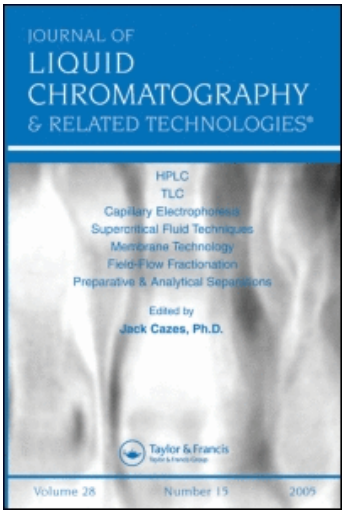

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Chlorhexidine Breakdown Products Including 4-Chloroaniline in Surgical Scrubs Containing Chlorhexidine

Minh Hoang[a]; John F. Moellmer[a]; Mohammad A. Khan[a]
[a] Deseret Medical, Inc., Utah

To cite this Article Hoang, Minh , Moellmer, John F. and Khan, Mohammad A.(1990) 'Separation of Chlorhexidine Breakdown Products Including 4-Chloroaniline in Surgical Scrubs Containing Chlorhexidine', Journal of Liquid Chromatography & Related Technologies, 13: 13, 2677 — 2687
To link to this Article: DOI: 10.1080/01483919008049062
URL: http://dx.doi.org/10.1080/01483919008049062

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(13), 2677-2687 (1990)

SEPARATION OF CHLORHEXIDINE BREAKDOWN PRODUCTS INCLUDING 4-CHLOROANILINE IN SURGICAL SCRUBS CONTAINING CHLORHEXIDINE

MINH HOANG, JOHN F. MOELLMER,
AND MOHAMMAD A. KHAN
Deseret Medical, Inc.
9450 South State Street
Sandy, Utah 84070

ABSTRACT

Chlorhexidine is an effective antimicrobial agent used in surgical hand scrubs and patient pre-operative preps for skin disinfection. As these solutions age, chlorhexidine degrades to produce breakdown products including 4-chloroaniline. The determination of 4-chloroaniline is usually carried out by a cleanup protocol using chlorinated solvents for sample preparation and by gas chromatography for analysis. This procedure is tedious and requires special precautions.[2] Current HPLC methods[3,4] are unable to analyze the chlorhexidine breakdown products in surgical scrub solutions which contain surfactants, gelling agents, etc., that give rise to complications in the resolution of peaks. The method reported here overcomes these problems while preserving the overall simplicity of the current HPLC methods. The 4-chloroaniline peak is clearly resolved from the peaks of the other breakdown products.

INTRODUCTION

Chlorhexidine and its salts, frequently chlorhexidine gluconate, has been a popular antimicrobial agent for many years since being first reported by Davis, et al.,[5] in 1954. Its broad spectrum

disinfectant activity against vegetative gram-positive and gram-negative bacteria has stimulated its inclusion into many medical products where reduction of disease causing micro-organisms has been intended.[6,7] Chlorhexidine digluconate is generally used to formulate the scrub and prep solutions, and several products under different trade names are currently in use. Chlorhexidine digluconate in specific concentrations in formulations has also been used in wound disinfection and as a mouth wash in the reduction of plaque formation and gingival inflammation.[8]

As chlorhexidine, its salts, and its solutions age, the chlorhexidine molecule will slowly degrade to form degradation products. This degradation of chlorhexidine is promoted by light, heat, and ionizing radiation. The breakdown product of greatest concern is parachloroaniline (PCA) because of its toxicity. There is then a great need to accurately determine the concentration of PCA particularly in medical products which, because of their age and storage conditions, may become more concentrated in this breakdown product. BP specification allows a limiting concentration of 500 p.p.m. in formulations. Solutions containing this amount of 4-chloroaniline are not known or suspected to be toxicologically hazardous.[1]

The British Pharmacopeia[9] uses a colorimetric method to determine the concentration of PCA in chlorhexidine and chlorhexidine salts, and does not lead to a numerical result. HPLC methods[3,4] are extant which show the determination of PCA in various solutions providing these solutions contain few surfactants, gelling and wetting agents, etc. However, surgical scrub solutions will contain these agents in relatively large concentrations compared to the PCA concentrations. Surfactants, for example, often are included

in a formulation in 1 to 20 percent concentrations. When currently available HPLC methods are attempted with surgical scrubs to determine the PCA concentration, severe column degradation occurs resulting from irreversible adsorption onto the column stationary phase. These problems appear in the form of changes of retention times, changes in peak areas, overlapping peaks and peaks missing altogether.

Because of these problems, the current method of determining PCA in surgical scrubs has been to first perform a cleanup protocol to remove as much of the surfactants as possible and then to inject samples into a GC.[2] However, in order to separate the PCA from the surfactants, the cleanup protocol uses methylene chloride and chloroform to form phases immiscible with water in several steps. By regulating the pH of the water phase and selecting the appropriate organic phase, separations are effected. This method of determining PCA in surgical scrubs is very tedious. Additionally, the several steps required in the cleanup protocol create the opportunity for the introduction of numerous errors.

A method has been developed to use HPLC analysis for determination of PCA in surgical scrub solutions in a relatively quick and safe manner which avoids the problems described above. It will be shown that this is a precise and accurate method which has wide application regardless of the use of various surfactants commonly in use among the manufacturers of surgical scrubs.

EXPERIMENTAL

A Waters Associates HPLC system was used which consisted of the following components: a model 480 Lambda-Max detector,

a model M730 Data Module, a model 6000A chromatography pump, a model 680 automated gradient controller, a model U6K Universal Injector, and a Nova-Pack C-18 3.9 mm x 30 cm column. A Hamilton #802 25 microliter syringe was used for all injections.

The various solvents used included HPLC grade methanol, reagent grade glacial acetic acid, HPLC grade isopropyl alcohol, HPLC grade acetonitrile, and distilled water from a Corning Mega-Pure Still System. The 1-pentanesulfonic acid was obtained from Eastman Kodak and the PCA (4-chloroaniline) was obtained from Aldrich at 98% pure and was used as received.

The injection volume used was 15 microliters and the detector wavelength set to 295 nm as determined from a Hewlett-Packard Model 9850 Diode Matrix Spectrophotometer. The detector sensitivity was set to 0.02 to 0.002 AUFS, as required. Peak width values were 35 to 40, noise rejection values 1 to 5, and area rejection value was 50, as required. The mobile phase was prepared by first mixing 30% acetonitrile, 30% methanol, and 40% distilled water. Next, 1-pentanesulfonic acid was added to effect a 0.005 M solution (0.96 gm/1), followed by sufficient glacial acetic acid to lower the pH to 3.5. The mobile phase was then vacuum filtered with a 5 micrometer polypropylene membrane.

The flow rate gradient program used was as follows: from 0 to 19 minutes, 0.3 ml/min; from 19 to 20 min, 0.6 ml/min; from 20 to 41 min, 1.0 ml/min, from 41 to 42 min, 0.3 ml/min; and the programmed run was terminated at 42 minutes. A single HPLC pump was used because the mobile phase was formulated to obviate the need for a second pump to change the mobile phase during the run program. Although a programmable multiple injector was not used

in this study, the flow rate gradient program would work well with one.

The standards were prepared by weighing 0.10 gm of PCA and carefully delivering it into a 1000 ml volumetric flask. The PCA was dissolved and the flask filled to the mark with a 1:1 mixture of isopropyl alcohol and water. A second sample was similarly prepared. The approximate concentration of these standards is 100 ppm. Dilutions were prepared with the appropriate pipets and volumetric flasks and IPA/water mixture to prepare standards of concentrations of 10 ppm, 5 ppm, 1 ppm, and 0.1 ppm. The samples were prepared by weighing approximately 10.0 grams of bulk solution into a 100 ml volumetric flask and filling the flask to the mark with distilled water.

The standards and samples are injected into the HPLC, insuring that the samples' concentrations of PCA fall within the range of the prepared standards. The determination of the PCA concentration in the injected samples is made by preparing a curve of standard peak areas versus their respective concentrations. The areas from the PCA in the injected samples are then compared with the curve to determine concentration in the samples. A linear regression algorithm will easily accomplish this in a calculator or computer.

RESULTS AND DISCUSSION

Chromatograms were obtained from the injection of standards of concentrations of 0.1 ppm, 1.0 ppm, 5.0 ppm and 10.0 ppm PCA. The areas subtended by the elution curve were then plotted against the concentrations of the samples. A linear regression of the

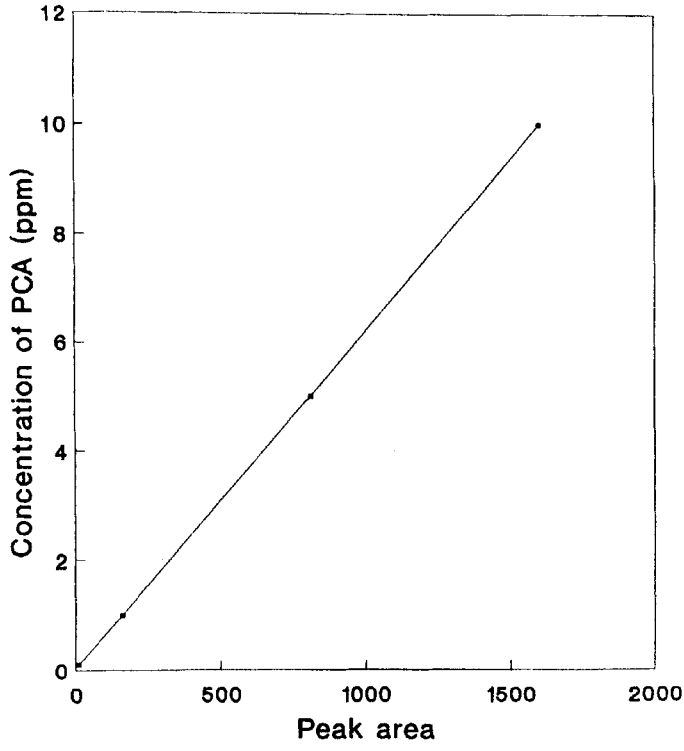

Figure 1. Calibration Curve of Peak Area Vs. Concentration of 4-Chloroaniline

above data shows a correlation coefficient of 0.99996. Figure #1 shows a least squares generated fitted line.

In order to ensure that the response of the instrumental method is linear with regard to the concentration of PCA in the surgical scrub, a sample of Manufacturer A brand chlorhexidine gluconate surgical scrub was studied in depth. First, the sample was prepared, injected and analyzed for its PCA concentration.

TABLE NO. 1

Spiked PCA Concentration in Brand A Sample

PCA Conc. In The Sample (PPM)	Spiked Conc. Of PCA (PPM)	Total Conc. Of PCA (PPM)	Measured Conc. Of PCA (PPM)
0.8	0.5	1.3	1.2
0.8	2.5	3.3	3.6
0.8	5.0	5.8	5.2

Next, serial additions of PCA were made to this sample ("spiking") to insure that the instrumental method was correctly measuring the PCA concentration known to exist in the sample. The "nonspiked" sample was first run to determine its PCA, then three additional samples were obtained from the first with the PCA concentrations deliberately set as shown in Table No. 1. The measured concentration of PCA is also recorded.

Because each manufacturer of chlorhexidine based surgical scrubs will formulate according to his own philosophy, there is a high likelihood that some surfactants will cause interference with the chromatographic method in some marketed products and not in others. Therefore, several manufacturers' products were examined for resolution of the PCA peak and linearity of response to PCA concentration. The analysis of these surgical scrub solutions was carried out in the following manner: First, a sample of the surgical scrub was run and compared to the standards which had been prepared. Once the concentration of PCA had been determined,

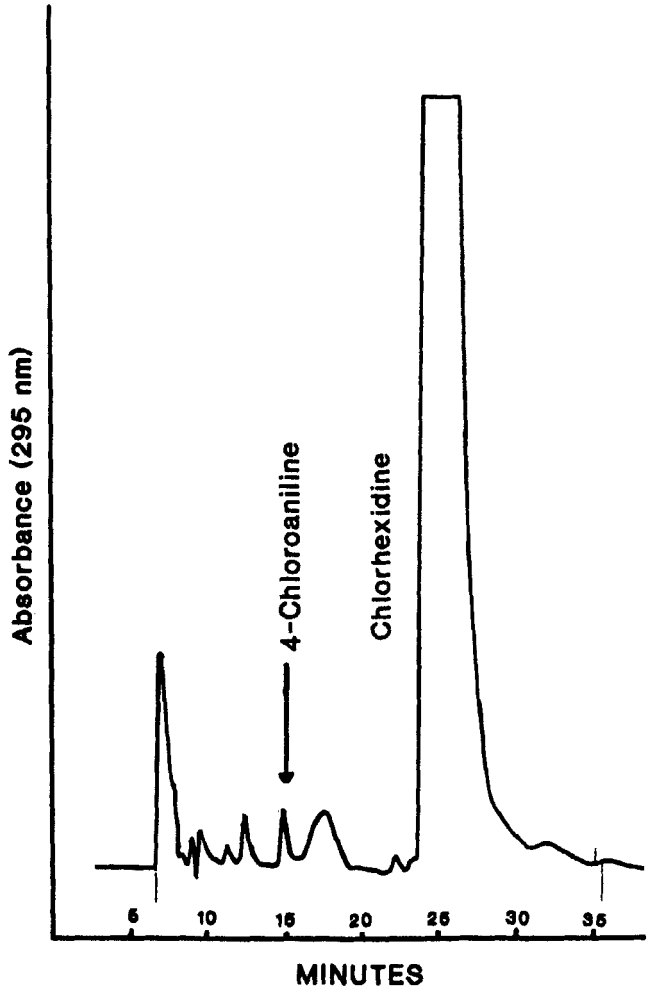

Figure 2. Typical Chromatogram for the Resolution of the 4-Chloroaniline Peak in Brand A Chlorhexidine Gluconate Surgical Scrub Solution

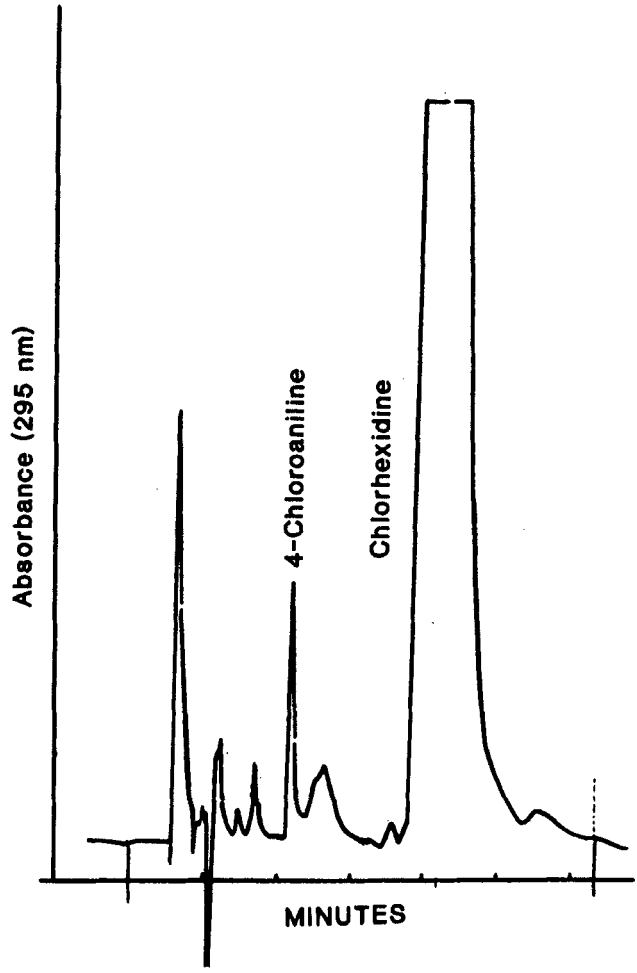

Figure 3. Chromatogram for the Resolution of the 4-Chloroaniline Peak in Brand A Spiked Chlorhexidine Gluconate with 4-Chloroaniline

TABLE NO. 2

Spiked Concentration of PCA in Different Brands of Samples

Brands	PCA Conc. In The Sample (PPM)	Spiked PCA Conc. In The Sample (PPM)	Total Conc. Of PCA (PPM)	Measured Conc. Of PCA (PPM)
A	0.8	5	5.8	5.2
B	0.85	5	5.85	5.9
C	0.55	5	5.55	5.2
D	1.05	5	6.05	5.9
E	0.75	5	5.75	5.8
F	1.2	5	6.2	6.8

then a known amount of PCA was additionally added to the surgical scrub and the "spiked" sample was rerun. The validity of the methodology was then determined by seeing if the concentration of PCA in the "spiked" sample was indeed the amount mathematically predicted.

Six brands of surgical scrubs (Brands A, B, C, D, E and F) were studied. Figure 2 shows a typical chromatogram for the resolution of the PCA peak. Note that the PCA peak is well resolved. In order to confirm that the PCA peak is correctly identified and that the instrumental response to the various formulations is still linear, the addition of a PCA "spike" was made to each manufacturer's sample and reinjected. Figure 3 shows that the PCA peak is properly identified and that the instrumental method is correctly determining the concentration of PCA. The results are shown in Table No. 2.

The instrumental precision of the methodology was checked by repeated injection of one sample. Ten sequential injections of the Brand A surgical scrub spiked with 5.0 ppm PCA was examined for the consistency of PCA concentration as determined by the chromatographic area. The mean and the standard deviations were calculated for the spiked concentration of PCA. The average concentration of PCA was found to be 5.15 ppm ± 0.10 ppm. The data indicates the results are reproducible.

REFERENCES

(1) A. E. Scott and E. Eccleston, Proc. Bur. Soc. Stnd. Drug Toxicity, 1966, 8, 195-204.

(2) U.S. NDA No. 18-423.

(3) R. L. Perez, J. Chromatogr. Sci., 1981, 19, 570-572.

(4) A. Richard, M. Elbaz and G. Andermann, J. Chromatogr, 1984, 298, 356-359.

(5) G. E. Davis, J. Francis, A. R. Martin, F. L. Rose, and G. Swain, Br. J. Pharmacol., 1954, 9, 192-196.

(6) N. Senior, J. Soc. Cosmet. Chem., 1973, 24, 259.

(7) D. G. Higgins, Chemist Drugg., 1974, 201, 518-521.

(8) M. A. Bassiouny and A. A. Grant, Br. dent. J., 1975, 139, 323-327.

(9) British Parmacopoea, 1973, Her Majesty's Stationary Office, London, England, 1973, p. 98.