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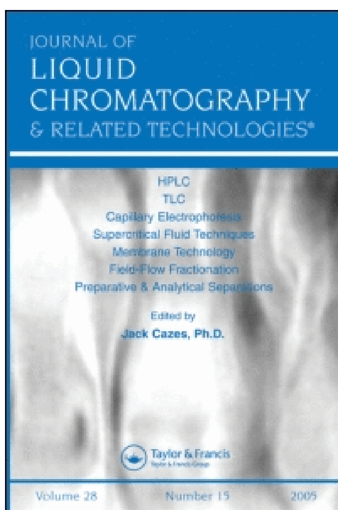

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Analysis of Coloured Chlorhexidine Solutions by Ion-Pairing Reverse-Phase High Pressure Liquid Chromatography

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ANALYSIS OF COLOURED CHLORHEXIDINE SOLUTIONS BY ION-PAIRING REVERSE-PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

An isocratic, high pressure liquid chromatographic procedure is described for the analysis of mixtures of chlorhexidine acetate and tartrazine, carmoisine or methylene blue. The method uses an ion-pairing reverse-phase technique which can be performed in less than 30 minutes.

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Chlorhexidine (1,6-di(N-p-chlorophenyldignanido)hexane), a strongly basic compound which forms salts with acids, was first described by Davis et al (1) in 1954. Originally, its chief use was as a preservative in ophthalmic solutions. In a subsequent study of the antibacterial properties of chlorhexidine (2), it was shown to have high static and cidal activity against a variety of bacteria and fungi exceeding the activity of benzalkonium chloride, and is now used as a contact antiseptic.

Three main solutions of chlorhexidine are currently manufactured in this plant and, as an aid to nursing staff working in busy situations, each of the three solutions is differently coloured (i.e. colour coded) for ease of identification and selection. The three main solutions manufactured are:

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1. Aqueous Chlorhexidine Acetate containing methylene blue as colourant.
2. An Aqueous mixture of Chlorhexidine Acetate and Cetrimide containing tartrazine (CI Food Yellow 4) as the colourant.
3. A 70% Ethanolic solution of Chlorhexidine Acetate coloured with carmoisine (CI Food Red 3).

The methods described in British Pharmacopoeia (3) and the British Pharmaceutical Codex (4) for the analysis of chlorhexidine containing products are not readily adaptable to solutions of chlorhexidine containing weak acids or bases (such as dyes), and it was necessary to develop an alternative analytical method for the analyses of the above solutions. In addition, it was necessary to determine the dye content of the solutions.

As reverse-phase ion-pairing HPCL has been successfully used for the analyses of FD & C colours in various mixtures (5, 6), it was decided to develop a method of analysis based on this approach.

In this paper, a reverse-phase ion-pairing method is described which allows the separation and quantitative determination of both chlorhexidine and either methylene blue, tartrazine or carmoisine, separately or in combination. Each solution may be analysed in less than 30 minutes.

MATERIALS AND METHODS

Apparatus

The apparatus used consisted of a Varian Model 500 liquid chromatogram equipped with a Varian Vari Chrom variable UV-Visible detector. Injection was by means of a Valco injector valve with a 10 μl loop (P/N CU-6-UHPa-N60) from Valco and a Glenco 1.0 ml syringe from Glenco Scientific

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Inc., Houston, Texas. A Linear Instruments Model 300 Series recorder was coupled to the detector.

The column used was a Varian Micropak MCH-10, 30 cm x 4 mm (id) (P/N 03-912151-44) obtained from Varian P/L, Sydney, Australia.

Reagents & Standards

HPLC grade acetonitrile and a 0.25% solution of 1-pentanesulphonic acid in glacial acetic acid, and analytical grade phosphoric acid and triethanolamine were obtained from Ajax Chemicals, Sydney, Australia.

Individual dye standards were obtained from Hodgsons Dye Agencies Pty. Ltd., Sydney, Australia, and standard chlorhexidine acetate was obtained from Imperial Chemical Industries Pty. Ltd., Villawood, Australia.

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Solutions of chlorhexidine acetate and the various dyes were prepared in distilled water to provide the following concentrations: chlorhexidine acetate, 0.005%; methylene blue, 0.01%; carmoisine, 0.01%; tartrazine, 0.004%. These solutions were used for peak identification and also for the preparation of approximately 50:50 mixtures of chlorhexidine and the various dyes.

The mobile phase was prepared by adding 20 ml of a 0.25% solution of pentane sulphonic acid in glacial acetic acid, and 5 ml of triethanolamine to 350 ml of acetonitrile and making up to 1000 ml with water. This solution was then adjusted to pH 3.00 with phosphoric acid and degassed by vacuum.

CHROMATOGRAPHIC PROCEDURES

All chromatograms were carried out isocratically at room temperature (ca 20°C) and at 2.0 ml/min flow rate except as indicated in Fig 4. In each case the detector was set at 8nm slit width, 260 nm wavelength and 0.1 absorbance units full scale deflection. The recorder chart speed was 0.5cm/min. Each chromatogram represents a 10 μl injection.

RESULTS AND DISCUSSION

Figs 1, 2 and 3 illustrate the chromatograms obtained from mixtures consisting of approximately 0.0025% chlorhexidine acetate, with either 0.002% tartrazine, 0.005% carmoisine or 0.005% methylene blue respectively.

As seen from Fig 1, the elution volume of tartrazine is very close to the void volume of the system when using the mobile phase and flow rate described. Also, from Fig 3, it can be seen that at a flow rate of 2 ml/min the retention time of methylene blue is unnecessarily long and leads to peak broadening. This can be improved by separating and analyzing

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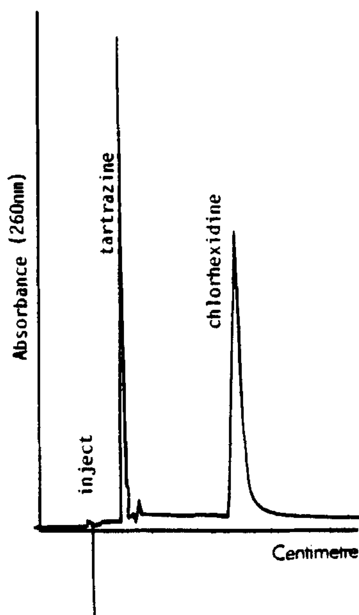

Figure 1. Chromatogram showing isocratic separation of chlorhexidine and tartrazine. Mobile phase and conditions as per Materials and Methods.

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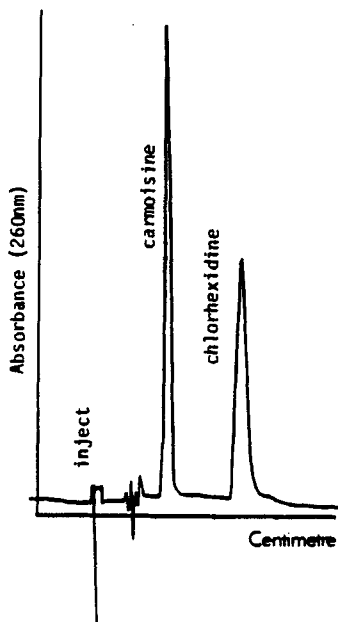

Figure 2. Chromatogram showing isocratic separation of chlorhexidine and carmoisine mobile phase and conditions as per Materials and Methods.

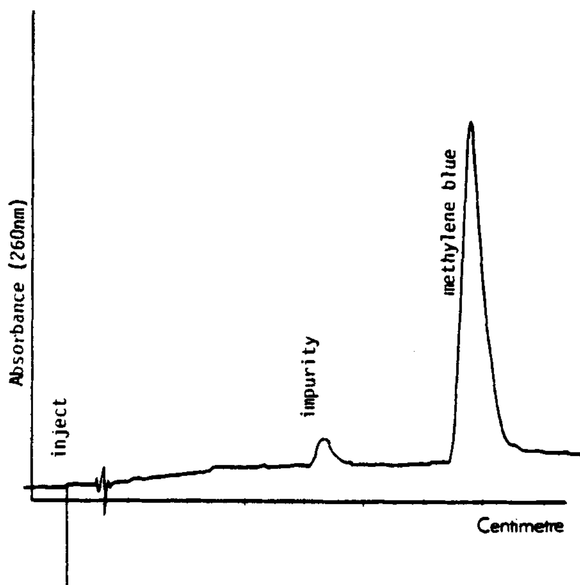

Figure 3. Chromatogram of methylene blue. Sample amount: 1.0 µg on column. Mobile phase and conditions as per Materials and Methods.

chlorhexidine acetate - methylene blue mixtures at a flow rate of 3.0 ml/min as in Fig 4 where the retention time for methylene blue becomes 4.4 cm (8.8 min).

The method here described has been used in this laboratory for the analysis of solutions containing chlorhexidine acetate in the range of 0.015% to 0.05% in the presence of 0.0002% of the said dyes with an accuracy of 3% for chlorhexidine and 5% for the dye component based on the physical measurement of peak height. The presence of cetrimide does not interfere with either the separation or quantitation of the chlorhexidine acetate - dye mixtures.

CONCLUSION

A method of analysing mixtures of chlorhexidine acetate - dye solutions is described here which allows the quantitative determination of these components in less than thirty

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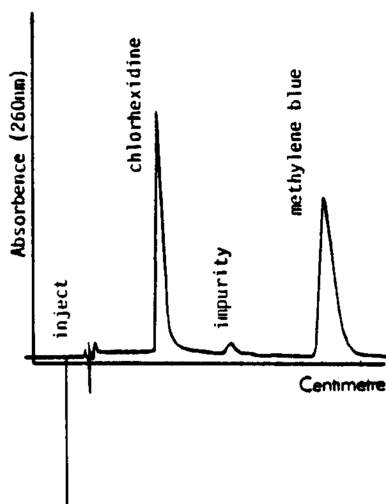

Figure 4. Chromatogram showing isocratic separation of chlorhexidine and methylene blue. Flow Rate: 3 ml/min. Other conditions as indicated under Materials and Methods.

minutes. This is to be compared with the non-specific and somewhat time consuming methods described in the British Pharmacopoeia (3) and the British Pharmaceutical Codex (4) for the determination of chlorhexidine.

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