Journal of Chromatography, 109 (1975) 305–312

CHROM. 8276

AUTOMATED CHROMATOGRAPHIC DETERMINATION OF CHLORHEXIDINE IN PHARMACEUTICAL PREPARATIONS

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(Received February 26th, 1975)

SUMMARY

An automated method for the determination of chlorhexidine (Hibitane) and its salts in formulated pharmaceutical products is described. The equipment consists of a high efficiency liquid chromatograph, a variable wavelength high sensitivity ultraviolet spectrophotometric detector, the output of which is monitored simultaneously by a suitable recorder, and a digital computer. The sample is automatically introduced on to a 10-cm silica gel column by use of a slide valve. Results are calculated and printed out by the computer.

INTRODUCTION

The assay of chlorhexidine (Hibitane*) formulations in current use in this laboratory requires extraction of the active agent from the formulation followed by a colourimetric procedure with alkaline hypobromite[1]. The procedure is accurate and selective but has the disadvantage in that it is time consuming, and as the complexity of the formulation increases so the sophistication of the required extraction procedure increases. The application of high-efficiency liquid chromatography in pharmaceutical analysis has already been described[2]. Recent advances in the technology of packing materials (particularly alumina and silica) have made possible the separation of chlorhexidine from its synthesis and degradation products. This separation is utilised to provide a specific analysis of the concentration of chlorhexidine and its salts in formulated products.

EXPERIMENTAL

The basic instrumentation of the automated liquid chromatograph comprises the following parts: coil pump; 10-cm glass column (4 mm internal diameter, 6.5 mm external diameter) of 11-μ silica gel (Partisil) packed by a modified tap procedure; "Servomex SV220" slide valve; "Cecil" sample changer; "Cecil 212 UV monitor";

* The word "Hibitane" is a trade mark, the property of Imperial Chemical Industries Limited.

suitable recorder; "Datachrom" computer system; the Interface is described in Fig. 1.

The coil pump supplies eluent phase at column pressure to the injection valve by way of a cut off valve activated by either a power failure or by a digital output from the computer, the digital output operating a relay switching the electrical supply to the valve. A manual override is fitted into this circuitry to enable the chromatograph to be operated manually if required. The slide valve has two solvent (5 μl) passages and is arranged such that the eluent phase can flow through one whilst the sample passes through the other, injection being achieved by switching the slide valve by pneumatic controller operated by means of a signal switched from the timer unit of the Cecil sample changer. The Cecil sample changer is switched by means of an optical coupler operating on a digital output from the computer, capability for manual override being retained. The computer is then notified of injection by means of a signal from the timer unit of the Cecil.

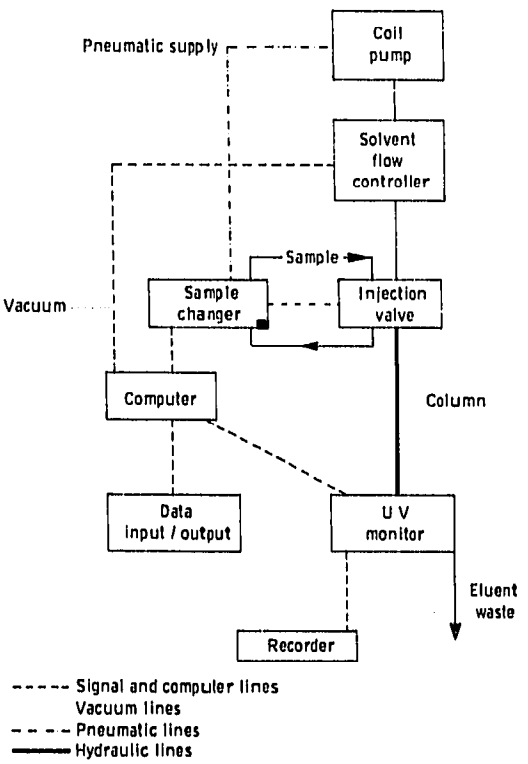


Fig. 1. Schematic diagram of automated high-pressure liquid chromatograph.

The chromatogram resulting from the separation on the column and the subsequent detection of the chlorhexidine by its ultraviolet absorption is monitored by both the recorder and the Datachrom computer system. The chromatograms (Figs. 2 and 3) show the separation of chlorhexidine from excipients in lotion and patch testing kits, demonstrating the degree of selectivity obtainable and enabling the chlorhexidine concentration in the samples to be calculated by comparison of the

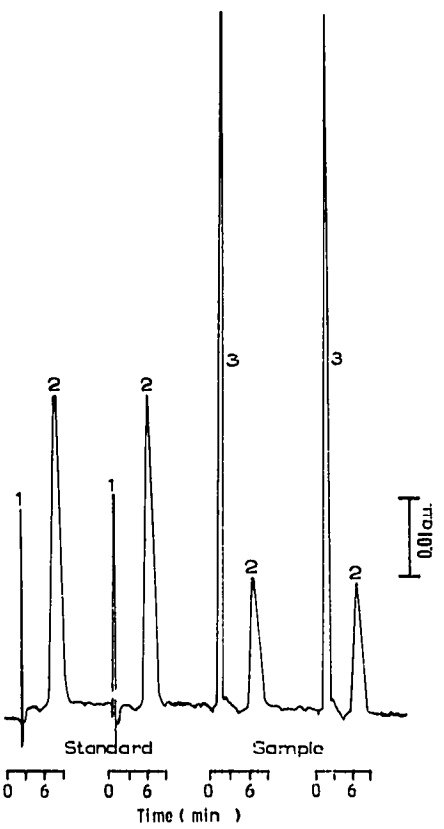
height of the chlorhexidine peak of the sample with that of a standard of known concentration.

The computer analyses the data acquired from the monitoring of the sample chromatogram according to the computer method. The acquired data are then referenced to the data acquired during the chromatographic run of a standard and the composition of the sample calculated in accordance with the computer method. The results are then presented to the operator as a printout.

Linearity of response with changes of concentration was checked over the range 0–600 μg/ml using solutions of chlorhexidine in methanol (Fig. 4).

Standard preparation

Transfer an accurately weighed quantity of standard chlorhexidine diacetate (50 mg) to a 200-ml flask, dissolve in and make to volume with methanol.



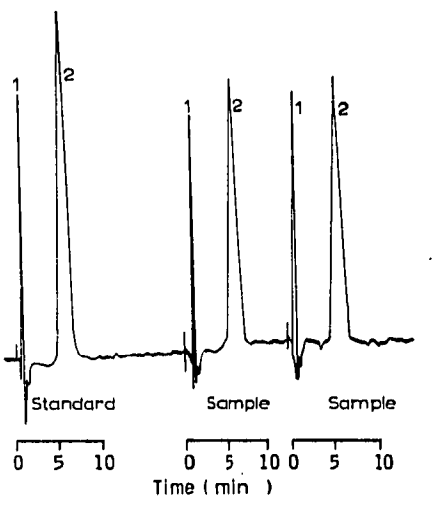


Fig. 2. Chromatogram of chlorhexidine standard and an extract of Savlon baby lotion. 1 = Solvent; 2 = chlorhexidine; 3 = excipient and solvent.

Fig. 3. Chromatogram of chlorhexidine standard and a sample from a patch testing kit. 1 = Solvent; 2 = chlorhexidine.

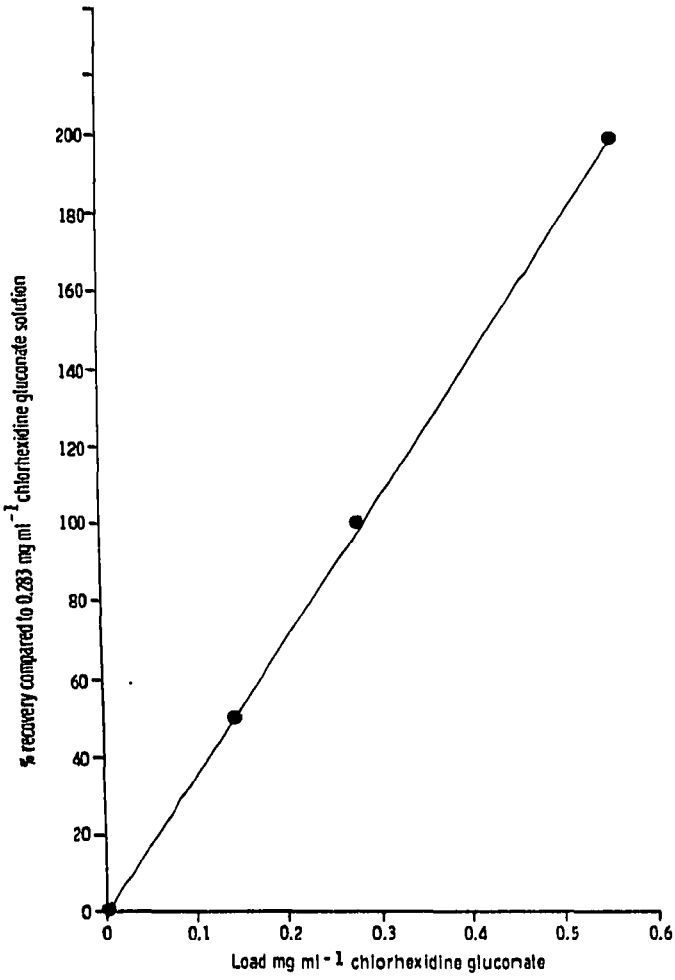


Fig. 4. Graph demonstrating linearity of chromatograph response to chlorhexidine.

Preparation of typical sample solutions

Savlon liquid antiseptic.* Transfer 10.0 ml of the sample to a 100-ml flask, dilute and make to volume with methanol.

Savlon hospital concentrate. Transfer 2.0 ml of sample to a 100-ml flask, dilute and make to volume with methanol.

Hibitane dental gel. Transfer an accurately weighed quantity of sample (1 g) to a 100-ml flask, disperse and make to volume with methanol. Use the supernatant solution.

Hibitane medical concentrate. Transfer 1.0 ml of the sample to a 200-ml flask, dilute and make to volume with methanol.

* The word "Savlon" is a trade mark, the property of Imperial Chemical Industries Limited.

*Hibiscrub**. Transfer an accurately weighed quantity (1 g) of sample to a 100-ml flask, dilute and make to volume with methanol.

Savlon antiseptic lozenges. Determine the average weight of a lozenge. Grind the lozenges to a fine powder, transfer an accurately weighed quantity (2 g) of the powder to a 100-ml separating funnel. Add 0.1 *M* sodium hydroxide (50 ml) and shake the mixture until dispersed. Extract the sodium hydroxide with three successive 25-ml volumes of diethyl ether. Combine the diethyl ether washings and evaporate to dryness. Dissolve the residue in a 1% solution of 1,5-gluconolactone in methanol, transfer this solution to a 25-ml flask and make to volume.

Savlon baby talc. Transfer an accurately weighed quantity (5 g) of sample to a 50-ml flask, disperse and make to volume with methanol. Shake the flask vigorously for 5 min. Allow the dispersion to settle and use the supernatant solution.

Savlon babycare lotion. Transfer an accurately weighed quantity (5 g) of sample to a separating funnel, disperse the sample in 25 ml of methanol, add 25 ml of isooctane and shake vigorously. Run the lower methanolic layer into a second separating funnel, and re-extract with a further 25 ml of isooctane. Transfer the methanolic layer to a 50-ml flask and make to volume with methanol.

Hibitane obstetric cream. Use the procedure as for Savlon babycare lotion with a sample weight of about 1 g and adjust the final volume to 100 ml.

Chromatography

The conditions for chromatography were as follows: column, 10 cm, glass, packed with 11-μ silica gel "Partisil" by a modified tap procedure; eluent, acetonitrile (general-purpose reagent)–0.02 *N* sulphuric acid in water (Analar) (91.5:8.5); pressure, 300 p.s.i.; flow-rate, 1 ml/min; temperature, 25°; UV detector wavelength, 254 nm; UV detector attenuation, 0.1 a.u.f.s.

The sample solution, prepared as previously described, was transferred to the sample vials of the automatic sample changer in duplicate. A standard, prepared as previously described, was placed at the beginning of the run of samples, in the middle, and at the end of the run. Then the automatic chromatograph was initiated.

RESULTS AND DISCUSSION

The automated high-efficiency liquid chromatographic procedure has been applied to a range of production and development samples and gives results indistinguishable from the colourimetric procedure. Degradation products and other impurities in chlorhexidine are separated by the chromatographic column with the following retention times: chlorhexidine, 320 sec; methanol, 50 sec; 4-chloroaniline, 80 sec.

The precision of the proposed analytical procedure was checked on a sample of Savlon liquid antiseptic and the results are tabulated in Table I. Recovery experiments of the three most widely used formulations were carried out. The results are shown in Table II.

* The word "Hibiscrub" is a trade mark, the property of Imperial Chemical Industries Limited.

TABLE I

RESULTS OF A SERIES OF REPLICATE ANALYSES ON A SAMPLE OF LIQUID ANTISEPTIC

Chlorhexidine gluconate (%, w/v)	*Mean (%)*	*Variance*
0.327	101.7	2.89
0.320	99.7	0.09
0.320	99.7	0.09
0.317	98.8	1.44
0.327	101.7	2.89
0.321	100.0	0.00
0.314	97.8	4.84
0.328	102.2	4.84
0.323	100.6	0.36
0.317	98.8	1.44

Standard deviation, 1.45%
Mean, 0.321% (w/v) chlorhexidine gluconate

TABLE II

RECOVERY OF CHLORHEXIDINE FROM PLACEBO FORMULATIONS

Sample	*Chlorhexidine gluconate (%, w/v)*		*Recovery (%)*
	Added	*Found*	
Savlon liquid antiseptic	0.282	0.283	100.4
Savlon hospital concentrate	1.50	1.48	98.9
Hibiscrub	3.95	3.94	99.8

TABLE III

COMPARATIVE ANALYSES OF SAVLON LIQUID ANTISEPTIC

Sample No.	*Chlorhexidine gluconate (%, w/v)*	
	Colourimetric result	*Chromatographic result*
1	0.31	0.32
2	0.32	0.33
3	0.32	0.32
4	0.32	0.31
5	0.32	0.32
6	0.32	0.33
7	0.33	0.31
8	0.31	0.30
9	0.32	0.30
10	0.31	0.29
11	0.32	0.31
12	0.32	0.32

TABLE IV

COMPARATIVE ANALYSES OF SAVLON HOSPITAL CONCENTRATE

Sample No.	*Chlorhexidine gluconate (%, w/v)*	
	Colourimetric result	*Chromatographic result*
1	1.52	1.52
2	1.56	1.54
3	1.51	1.50
4	1.50	1.49
5	1.59	1.57
6	1.36	1.36
7	1.50	1.53

TABLE V

COMPARATIVE ANALYSES OF SAVLON BABYCARE TALC

Sample No.	*Chlorhexidine hydrochloride (%, w/w)*	
	Colourimetric result	*Chromatographic result*
1	0.19	0.19
2	0.19	0.19
3	0.18	0.18

TABLE VI

COMPARATIVE ANALYSES OF HIBITANE MEDICAL CONCENTRATE

Sample No.	*Chlorhexidine gluconate (%, w/v)*	
	Colourimetric result	*Chromatographic result*
1	5.2	5.10
2	5.4	5.42
3	5.4	5.08

TABLE VII

COMPARATIVE ANALYSES OF HIBISCRUB

Sample No.	*Chlorhexidine gluconate (%, w/v)*	
	Colourimetric result	*Chromatographic result*
1	3.96	4.10
2	4.02	4.08

TABLE VIII

COMPARATIVE ANALYSES OF SAVLON BABYCARE LOTION

Sample No.	*Chlorhexidine gluconate (%, w/w)*	
	Colourimetric result	*Chromatographic result*
1	0.10	0.10
2	0.10	0.10
3	0.10	0.10
4	0.10	0.10

TABLE IX

COMPARATIVE ANALYSES OF MISCELLANEOUS CHLORHEXIDINE CONTAINING FORMULATIONS

Nature of sample	*Colourimetric result*	*Chromatographic result*
Hibitane obstetric cream	1.00% (w/w) Chlorhexidine gluconate	1.03% (w/w) Chlorhexidine gluconate
Hibitane acetate solution	0.10% (w/v) Chlorhexidine acetate	0.10% (w/v) Chlorhexidine acetate
Hibitane antiseptic lozenges	5.16 mg Chlorhexidine acetate/lozenge	5.30 mg Chlorhexidine acetate/lozenge
Hibitane dental gel	0.97% (w/w) Chlorhexidine gluconate	0.97% (w/w) Chlorhexidine gluconate

The results of comparative assays between the colourimetric and chromatographic procedures on a variety of formulations are given in Tables III–IX. The results demonstrate the wide applicability of the automated chromatographic procedure to the analysis of chlorhexidine containing formulations.

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