

# HPLC determination of chlorhexidine in dosage forms

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The present paper describes the utilization of high-pressure liquid chromatography for determination of chlorhexidine in complex samples of dosage forms. Chromatographic separation was performed on a cyanopropyl column using acetate buffer in the mixture of water and acetonitrile ( $\phi_r = 2:3$ ) as the mobile phase. The linear dependence of the peak size on concentration of chlorhexidine in the range of 0.01 to 0.20 mol m<sup>-3</sup> was ensured only when the samples were diluted with the mobile phase. Otherwise, the reproducibility of measurements decreased substantially.

В работе описывается применение жидкостной хроматографии под высоким давлением для определения содержания хлоргексидина в сложных образцах лекарственных форм. Хроматографическое разделение проводилось на колонне с цианопропилом, используя ацетатный буферный раствор в смеси воды и ацетонитрила ( $\phi_r = 2:3$ ) в качестве подвижной фазы. Линейная зависимость площади пика от концентрации хлоргексидина в диапазоне от 0,01 до 0,20 моль м<sup>-3</sup> обеспечивалась только когда образцы были разбавлены подвижной фазой. В ином случае воспроизводимость результатов значительно ухудшалась.

Determination of 1,6-bis[5-(4-chlorophenyl)biguanidino]hexane (further chlorhexidine), generally used as acetate or gluconate, has been provided by various methods. The most general of them is titration of chlorhexidine with perchloric acid in anhydrous medium, however, it is unusable in the presence of other basic components in the sample. Titration with sodium tetraphenyl borate in acetate buffer appears to be a selective method [1] but the results are too dependent on composition of the sample with regard to slow formation of the complex of tetraphenyl borate with chlorhexidine. It was found that in the presence of hydroxyalkylcellulose the titration could not be performed.

Widely used are various spectral methods based on colour reactions of chlorhexidine itself or its decomposition product, 4-chloroaniline [2—10]. These methods are practically not usable for determination of chlorhexidine in coloured solutions and, moreover, their reliability is very low. Application of the polarographic method [11] to determination of the pharmaceutical preparations studied herein has not been successful either.

For determination of chlorhexidine in such samples suitable are only separa-

tion methods. The decomposition product 4-chloroaniline has usually been established by gas chromatography [12–14]. However, the preparation of the sample for analysis is rather laborious. Chlorhexidine may be determined directly by thin-layer chromatography, however, its sensitivity is mostly insufficient [15, 16]. The most advantageous method appears to be high-pressure liquid chromatography on a sorbent of the RP-18 type, using a mobile phase composed of a mixture of water and acetonitrile or methanol, containing ion-pairing compounds (*e.g.* heptanesulfonic acid, pentanesulfonic acid, pentafluorooctanoic acid, *etc.*) [17–25]. These methods require time-consuming saturation of chromatographic columns and their reproducibility with the samples of pharmaceutical preparations analyzed herein was not satisfactory with regard to the disturbing influence of other compounds present.

The present paper describes a reliable liquid-chromatographic method for determination of chlorhexidine on a column with a silica gel-type sorbent containing cyanopropyl groups.

## Experimental

### *Instruments and equipments*

An SP 8700 liquid chromatograph, an SP 8440 XR spectrophotometric detector with variable wavelength, an SP 4200 integrator (all from Spectra Physics, U.S.A.), a syringe loading injector 7125 (Rheodyne, U.S.A.) with a  $10^{-5}$  dm<sup>3</sup> loop, and a glass chromatographic column (150 mm × 3 mm) packed with Separon SIX CN sorbent of 5 μm grain size (Laboratorní přístroje, Prague) were used for measurements.

### *Chromatographic conditions*

The mobile phase was prepared by dissolving sodium or potassium acetate, anal. grade (0.1 mol) and acetic acid, anal. grade (0.1 mol) (all from Lachema, Brno) in a mixture (1 dm<sup>3</sup>) of deionized distilled water and acetonitrile for HPLC (Fluka, Switzerland) in the volume ratio of  $\varphi_r = 2 : 3$ . Flow rate of the mobile phase was  $0.5 \text{ cm}^3 \text{ min}^{-1}$ , wavelength of radiation of the detector 235 nm, and sensitivity 0.04 absorbance units. The calibration solutions were prepared by dissolving chlorhexidine diacetate (ICI, Belgium) in the mobile phase. All samples of dosage forms analyzed were diluted with the mobile phase in the volume ratio of 1 : 5 at the least. The retention time of chlorhexidine was 5.8 min.

## Results and discussion

The peak sizes of chromatograms of chlorhexidine have been measured. Concentrations of chlorhexidine solutions in the mobile phase were 0.0168,

0.0358, 0.0793, 0.120 or 0.171 mol m<sup>-3</sup>; each solution was injected twice. The chromatograms are presented in Fig. 1. The dependence of the peak size on chlorhexidine concentration (calibration curve) was linear with the correlation coefficient 0.9997 (calculated by the least-squares method) and passed through the origin.

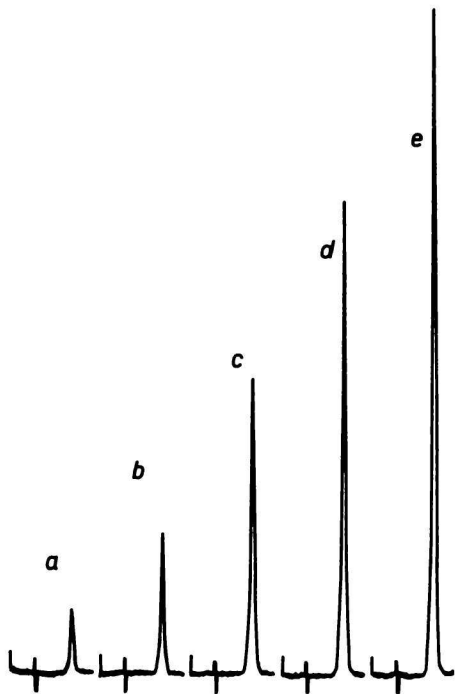


Fig. 1. Chromatograms of standard solutions of chlorhexidine diacetate dissolved in the mobile phase.

a)  $c = 0.0168 \text{ mol m}^{-3}$ ; b)  $c = 0.0358 \text{ mol m}^{-3}$ ;  
 c)  $c = 0.0793 \text{ mol m}^{-3}$ ; d)  $c = 0.120 \text{ mol m}^{-3}$ ;  
 e)  $c = 0.171 \text{ mol m}^{-3}$ .



Fig. 2. Chromatogram of three injections of the same solution of chlorhexidine (X) dissolved in methanol ( $c = 0.0319 \text{ mol m}^{-3}$ ).

The mode of the sample dilution in determination of chlorhexidine by the method described is a significant factor. Fig. 2 illustrates the chromatograms of three injections of the same chlorhexidine sample dissolved in methanol. The concentrations calculated from the calibration curve were 0.0793, 0.0309 or 0.0438 mol m<sup>-3</sup>. Results with a similar dispersion variance were observed when chlorhexidine was diluted with water or a mixture of water and methanol or acetonitrile.

The peak sizes of chromatograms of chlorhexidine solutions in methanol at 0.0080, 0.0158, 0.0319, 0.0477, 0.0798, 0.112 or 0.160 mol m<sup>-3</sup> concentrations were also measured. Each solution was injected thrice. The straightline obtained by plotting these values using the least-squares method did not pass through the origin and the scatter of peak size values of chlorhexidine in the concentration range of 0.008 mol m<sup>-3</sup> to 0.1 mol m<sup>-3</sup> was above 50 %. The reasons for this phenomenon are not known thus far.

Characteristic chromatograms of samples diluted with the mobile phase are presented in Fig.3 and Table 1 brings the results of determination of eight samples. From these results it follows that the method presented above for the determination of chlorhexidine is suitable for the given samples.

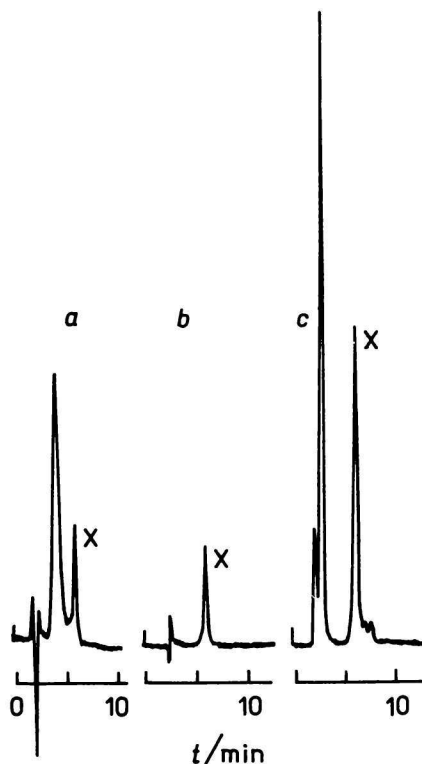


Fig. 3. Chromatograms of various samples of pharmaceutical preparations diluted with the mobile phase. a) The sample contains 0.056 mol m<sup>-3</sup> chlorhexidine, perfume, ionogenic tensides, dyes, and solvents; dilution 1 : 5. b) The sample contains 0.112 mol m<sup>-3</sup> chlorhexidine, acetate buffer, stabilizing additives, and solvents; dilution 1 : 10. c) The sample contains 5.57 mol m<sup>-3</sup> chlorhexidine, ionogenic tensides, dyes, stabilizing additives, and vehicle; dilution 1 : 50. The chlorhexidine peak is marked by X.

Table 1

Determination of chlorhexidine content in pharmaceutical preparations

Sample	Dilution	Number of analyses <sup>a</sup>	$c/\text{mol m}^{-3}$	
			Declared	Determined <sup>b</sup>
1	1:10	5	0.5	$0.485 \pm 0.012$
2	1:10	5	0.5	$0.501 \pm 0.010$
3	1:5	4	0.1	$0.090 \pm 0.003$
4	1:5	4	0.1	$0.092 \pm 0.004$
5	1:50	5	2.5	$2.46 \pm 0.04$
6	1:50	5	2.5	$2.47 \pm 0.02$
7	1:10	5	1	$1.01 \pm 0.018$
8	1:10	5	1	$0.99 \pm 0.020$

a) Number of analyses means the number of injections of the respective sample. b) 95% confidence limit was calculated by using the Student distribution.

For determination of chlorhexidine in very complicated samples, such as liquid dosage forms, only a selective method, e.g. high-pressure liquid chromatography, is suitable. Reproducible results may be obtained when some essential conditions are kept, namely, acetate buffer should be used as the mobile phase, the samples should be diluted with or dissolved in the same medium, and, finally, a silica gel sorbent with bound cyanopropyl groups should be used.

It has been verified that the method suggested provides reproducible results in all cases, provided that the sample is diluted with the mobile phase in the volume ratio of 1:5 at the least.

## References

1. Pinzanti, S. and La Porta, E., *Pharm. Acta Helv.* 56, 155 (1981).
2. Holbrook, A., *J. Pharm. Pharmacol.* 10, 370 (1958).
3. Goodal, R. R., Goldman, J., and Woods, J., *Pharmacol. J.* 200, 33 (1968).
4. Crooper, E., Platt, P., and Puttnam, N. A., *J. Soc. Cosmet. Chem.* 26, 355 (1975).
5. Lowry, J. B., *J. Pharm. Sci.* 68, 110 (1979).
6. Andermann, G., Bahler, M. O., and Erhart, M., *J. Pharm. Sci.* 69, 215 (1980).
7. van de Vaar, F. J., Holshoff, A., and Indemans, A. W., *Pharm. Weekbl., Sci. Ed.* 2, 179 (1980).
8. Pinzanti, S., La Porta, E., Casini, M., and Betti, C., *Pharm. Acta Helv.* 57, 334 (1982).
9. Arzamastsev, A. P., Kovalenko, L. I., Popov, D. M., and Skuratovich, A. A., *Khim.-Farm. Zh.* 17, 247 (1983).
10. Sukiasyan, A. N., Kopylova, A. I., and Malysheva, L. F., *Khim.-Farm. Zh.* 18, 1271 (1984).
11. Jacobsen, E. and Glyseth, B., *Talanta* 22, 1001 (1975).
12. Seifert, K., Casgrande, D., and Silberman, H., *J. Chromatogr.* 109, 193 (1975).

13. Matsushina, H. and Sakuri, N., *Nipon Koshu Eisei Zasshi* 29, 135 (1982).
14. Miribel, L., Brazier, J. L., Comet, F., and Lecomte, D., *J. Chromatogr.* 268, 321 (1983).
15. Guven, K. G., *Eczaelik Bul.* 12, 111 (1970).
16. Dalrup, T., Susanto, F., and Mischalke, P., *Z. Anal. Chem.* 308, 413 (1981).
17. Collins, A. J., Campbell, A. T., and Bain, R., *J. Clin. Pharmacol.* 4, 205 (1979).
18. Stuber, B. and Mueller, H. K., *Pharm. Acta Helv.* 55, 171 (1980).
19. Bachner, J., Heinisch, G., and Matous, H., *J. High Resolut. Chromatogr. Chromatogr. Commun.* 4, 132 (1981).
20. Perez, R. L., *J. Chromatogr. Sci.* 19, 570 (1981).
21. Huston, C. E., Wainwright, P., Cooke, M., and Simpson, R., *J. Chromatogr.* 237, 457 (1982).
22. Lake, O. A. *et al.*, *Pharm. Weekbl., Sci. Ed.* 5, 15 (1983).
23. Bauer, M., Mailhe, L., Menard, V., and Rouanet, J. P., *J. Chromatogr.* 259, 360 (1983).
24. Bauer, M., Degade, C., and Mailhe, L., *J. Chromatogr.* 315, 457 (1984).
25. Richard, A., Elbaz, M., and Andermann, G., *J. Chromatogr.* 298, 356 (1984).

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