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# SINGLE-DOSE DETERMINATION OF SUPPOSITORIES CONTAINING PHENYLEPHRINE, LIDOCAINE AND BETAMETHASONE VALERATE BY REVERSED-PHASE ION-PAIR LIQUID CHROMATOGRAPHY

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SUMMARY

The power of reversed-phase ion-pair liquid chromatography in drug analysis is demonstrated by the development of a method for the separation and quantitative determination of phenylephrine, lidocaine, and betamethasone valerate in combination. The separation is achieved on Nucleosil 5 C8 as the stationary phase and with methanol–0.01 M sodium dihydrogenphosphate (7 : 3) containing 0.005 M sodium dodecane sulphonate as the mobile phase. The method allows fast and reliable analysis of the three drug substances, and it is used for single-dose determination of a suppository preparation.

## INTRODUCTION

In order to evaluate the content uniformity of low-dose preparations increasing attention is paid to the development of methods for single-dose determinations. For pharmaceutical preparations containing two or more active ingredients high performance liquid chromatography (HPLC) offers a convenient tool, making it possible to determine all components in a single procedure only. When handling suppositories the proportion between the amount of excipients and active ingredients is increased drastically compared to other solid dosage forms, and the present mixture containing both ionic and non-ionic compounds is further complicated. The active principles are the neutral component betamethasone valerate and the two amine salts lidocaine hydrochloride and phenylephrine hydrochloride.

Several modes of HPLC are suitable for the separation of amines including ion-exchange (Cox et al., 1976), adsorption (Jane, 1975), and ion-pair partition in the straight-phase (Persson and Karger, 1974) and reversed-phase (Sood, 1976; Lurie, 1977). The determination of corticosteroids such as betamethasone valerate using reversed-phase HPLC was reported by Bailey and Brittain (1973).

The application of reversed-phase ion-pair chromatography as an alternative to ion-

exchange chromatography for the separation of ionic compounds was first reported by Witmer et al. (1975) and by Knox and Jurand (1975). In 1976 Korpi et al. reported a further advantage when handling a mixture of ionic and non-ionic compounds. For such mixtures the separation of the non-ionic compounds is first established, and then by the addition of a suitable buffer and a counterion the retention of the ionic compounds is controlled to achieve a suitable separation of the total mixture, a principle utilized in the separation of the present mixture. Furthermore, the application of the method for content uniformity testing is described.

## MATERIALS AND METHODS

### *Preparation*

One suppository contains 2 mg of phenylephrine hydrochloride, 40 mg of lidocaine hydrochloride and 0.5 mg of betamethasone valerate. The base excipient is Massupol (lauric acid triglyceride). The total weight of one suppository is 1.75 g.

### *Chromatography*

A liquid chromatograph consisting of a Haskel model 26980 pump, a Cecil 212 spectrophotometer detector, and a Rheodyne 7120 injection valve with a 20 µl loop was used. Chromatograms were recorded on a Kipp and Zonen model BD-8 recorder and peak areas were integrated by means of a Hewlett–Packard 3370B electronic integrator.

All experiments were performed on a 15 cm long and 4.65 mm i.d. column packed as earlier described (Helboe and Thomsen, 1977) with Nucleosil 5 C8 support (Macherey Nagel, Düren, G.F.R.).

The mobile phase was 0.01 M sodium dihydrogenphosphate (pH 4.8) modified with 70% of methanol. Different concentrations of alkane sulphonates were added as counterion. All reagents used were of analytical grade and were obtained from E. Merck (Darmstadt, G.F.R.).

### *Test and standard solutions*

*Standard solution:* 10 mg of phenylephrine hydrochloride, 200 mg of lidocaine hydrochloride and 2.5 mg of betamethasone valerate, was dissolved in 100 ml of 70% methanol in 0.01 M sodium dihydrogenphosphate; 20 µl were injected.

*Test solutions:* one suppository or the stated amount of powdered suppositories was treated with 50 ml of warm (70°C) isooctane until total dispersion was achieved. After cooling the mixture was extracted by shaking for 2 min with 20 ml of 70% methanol in 0.01 M sodium dihydrogenphosphate. The lower layer was centrifuged at 3000 rpm for 10 min and 20 µl of the clear supernatant were injected.

## RESULTS AND DISCUSSION

### *Chromatography*

Using Nucleosil 5 C8 as the support, the suitable composition of the mobile phase was found to be about 70% methanol in water giving a capacity factor ($k'$) for betamethasone

TABLE 1

INFLUENCE OF pH ON THE RETENTION (k')

Chromatographic conditions: 70% of methanol in 0.01 M phosphate buffers; 0.005 M sodium dodecane sulphonate added.

| Drug substance | 0.01 M Phosphate buffer | | |
|---|---|---|---|
| | pH 2.3 | pH 4.8 | pH 9.1 |
| Phenylephrine | 0.88 | 0.80 | 0.51 |
| Lidocaine | 1.86 | 1.78 | 1.79 |
| Betamethasone valerate | 3.06 | 3.16 | 3.08 |

valerate of 3.6, thus the two amine salts might be placed previously in the chromatogram. As the counterion for the ion-pairing with the amines, alkane sulphonates were chosen. Having established the solid support and the modifier concentration, three main factors concerning the mobile phase are expected to affect the retention of the two bases (Sood, 1976): pH, counterion concentration, and the nature of the counterion.

The influence of pH on the retention of the three compounds is not pronounced (Table 1). However, buffering the aqueous phase is still essential otherwise the peak shape for the amines deteriorates seriously after a few injections. To obtained prolonged column life 0.01 M sodium dihydrogenphosphate was chosen.

A hyperbolic dependence of k' on the counterion concentration in reversed-phase ion-pair chromatography using neat aqueous solvents was shown by Horvath et al. (1977). Furthermore, the addition of organic solvents such as methanol to the mobile phase caused a curve which was broadened and flattened. The curves of phenylephrine and lidocaine in Fig. 1 show only the first part of this predicted hyperbolic course due to a restricted solubility of the dodecane sulphonate. For betamethasone valerate the k' decreased slightly for increasing couterion concentrations. This effect indicates that the addition of a detergent to the mobile phase besides having the action as an ion-pairing agent is increasing the elution strength. For the separation a counterion concentration of 0.005 M was used. At this concentration the retention of betamethasone valerate was influenced only to a minor extent.

The influence of the nature of the counterion on the retention is shown in Fig. 2. An increase in the carbon number and thereby in the lipophilic properties of the counterion causes an increase in the retention of the ion-pairs. A slight decrease in k' for betamethasone valerate was observed for the long carbon chain counterions. From Fig. 2 it was concluded that an optimal separation in a reasonable time can be achieved using dodecane sulphonate as the counterion.

The detection wavelength is selected from the absorption spectra of the three compounds (Fig. 3) at the same relative concentrations as in the suppositories. Both at 230 and 260 nm a reasonable absorbance was achieved for the two low-dose compounds. However, when detected at 230 nm the peak of phenylephrine was disturbed by excipients, whereas this was not the case at 260 nm; thus the latter was chosen as the detection wavelength (Fig. 4).

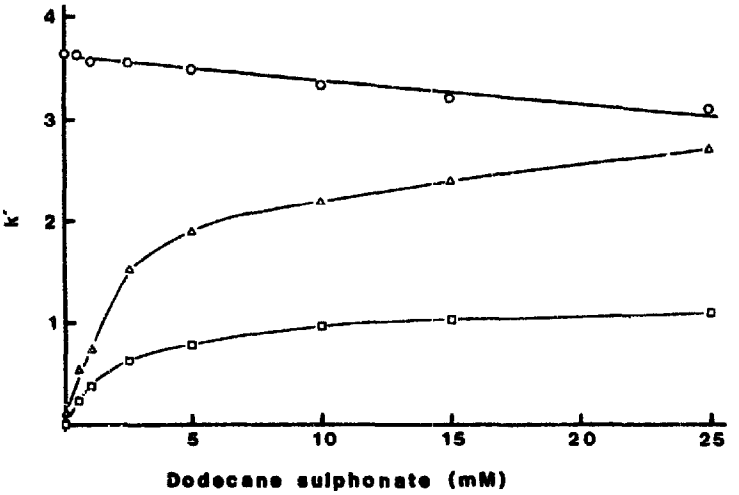


Fig. 1. Influence of counterion concentration on the retention. Chromatographic conditions: 70% methanol in 0.01 M phosphate buffer pH 4.8. Counterion: sodium dodecane sulphonate, □, phenylephrine; △, lidocaine; ○, betamethasone valerate.

*Extraction, recovery, linearity, and precision*

Most suppository excipients are fatty compounds which are strongly retained on a reversed-phase column, and even minor accumulation changes its characteristics. Accord-

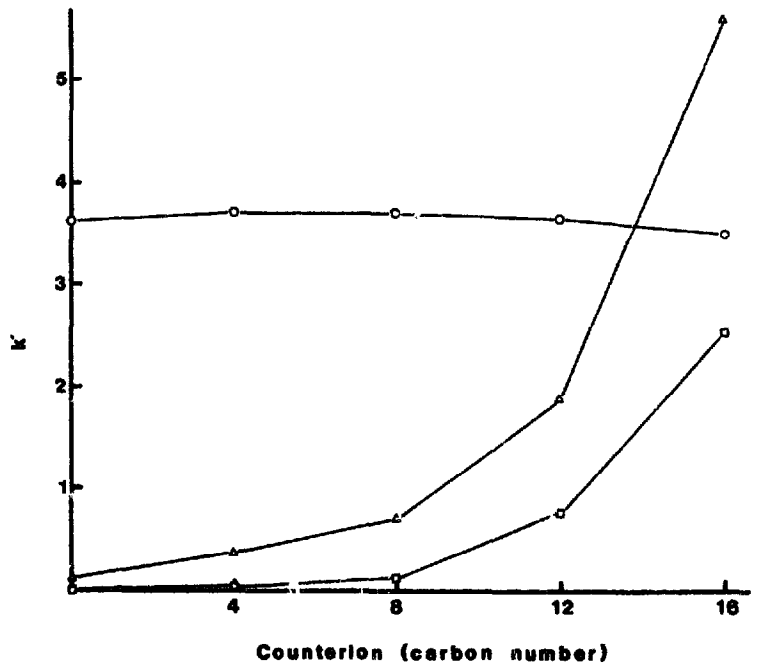


Fig. 2. Influence of the nature of counterion on the retention. Chromatographic conditions: 70% methanol in 0.01 M phosphate buffer pH 4.8. Counterion: alkane sulphonates. □, phenylephrine; △, lidocaine; ○, betamethasone valerate.

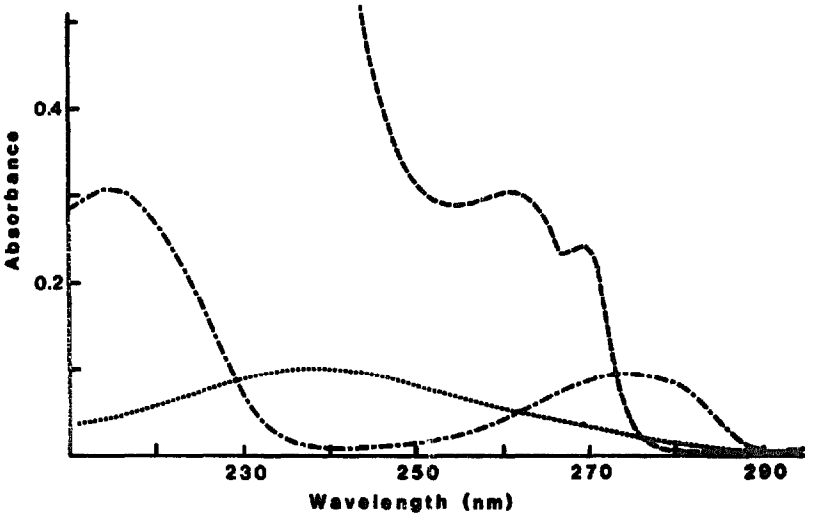


Fig. 3. UV absorption spectra of phenylephrine hydrochloride 10 μg/ml (· – · – ·), lidocaine hydrochloride 200 μg/ml (- - - - -), and betamethasone valerate 2.5 μg/ml (· · · · · ·). Solvent: 70% methanol in 0.01 M phosphate buffer pH 4.8.

ingly, in the analysis of suppositories by HPLC using the reversed-phase mode it is usually required to extract the active ingredients from excipients prior to chromatography. The present extraction was performed by solvent distribution of the compounds between

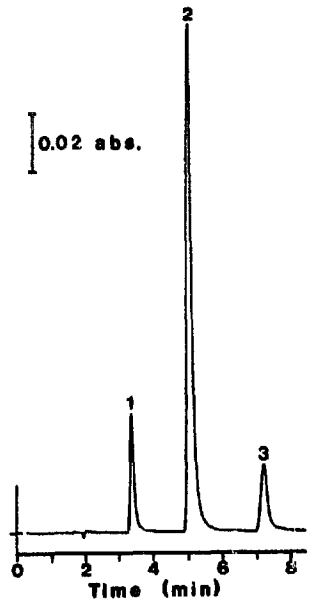


Fig. 4. Separation of active ingredients from suppositories. Support: Nucleosil 5 C8. Mobile phase: 0.005 M sodium dodecane sulphonate in methanol + 0.01 M phosphate buffer pH 4.8 (7 : 3). Solvent velocity: 1.5 mm/s. Pressure: 11 MPa. Detection wavelength: 260 nm. 1, phenylephrine hydrochloride, 2 μg (k' = 0.8); 2, lidocaine hydrochloride, 40 μg (k' = 1.9); 3, betamethasone valerate, 0.5 μg (k' = 3.6).

TABLE 2

RECOVERY OF THE METHOD

The stated recoveries are the result of duplicate performed analysis.

| Drug substance | Added amount mg | Recovery % |
|---|---|---|
| Phenylephrine hydrochloride | 0.5 | 103 |
| | 1.0 | 102 |
| | 2.0 | 102 |
| | 4.0 | 102 |
| | 7.0 | 100 |
| Lidocaine hydrochloride | 10.0 | 102 |
| | 20.0 | 102 |
| | 40.0 | 101 |
| | 80.0 | 100 |
| | 140.0 | 100 |
| Betamethasone valerate | 0.125 | 94 |
| | 0.25 | 97 |
| | 0.50 | 101 |
| | 1.00 | 102 |
| | 1.75 | 102 |

isooctane and 70% methanol in 0.01 M sodium dihydrogenphosphate. Using this procedure and detecting at 260 nm no disturbance due to excipients occurred.

The recovery and the linearity of the detector response were investigated at the same time. The three active ingredients in amounts corresponding to $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, and 4 suppositories, and in any case Massupol corresponding to one suppository, were extracted as mentioned above. The results of the HPLC analysis are shown in Table 2. The recovery of all three compounds in the range investigated was approximately 100%. Thus, satisfactory recovery as well as linearity of the method is demonstrated. From these results it was decided to perform the quantitative determinations using standard solutions in one concentration only. The precision of the method was examined by powdering 20 supposi-

TABLE 3

PRECISION OF THE METHOD

The means and the relative standard deviations are determined by the analysis of powdered suppositories. Each of the 10 extracts were chromatographed in triplicate.

| Drug substance | Label claim mg | $\bar{x}_{10}$ mg | $s_r$ % |
|---|---|---|---|
| Phenylephrine hydrochloride | 2 | 1.95 | 0.9 |
| Lidocaine hydrochloride | 40 | 38.39 | 1.1 |
| Bemathasone valerate | 0.5 | 0.53 | 1.3 |

TABLE 4

RESULTS OF SUPPOSITORY CONTENT UNIFORMITY TEST

Each of the 10 single dose extracts was chromatographed in triplicate. The content precision is corrected for analytical error (cf. Table 3).

| Drug substance | 10 assays within mg | $\bar{x}_{10}$ mg | Content precision $s_r$ % |
|---|---|---|---|
| Phenylephrine hydrochloride | 1.86– 1.97 | 1.93 | 1.5 |
| Lidocaine hydrochloride | 37.48–38.70 | 38.10 | 0.3 |
| Betametasone valerate | 0.52– 0.54 | 0.53 | 0.5 |

tories and analyzing amounts of powder corresponding to one suppository. The results are shown in Table 3.

*Content uniformity*

In a previous paper (Helboe and Thomsen, 1978) the tests for content uniformity included in most pharmacopoeias were referred to be based on fixed limits, although several authors have shown that more reliable tests should be based on the estimation of variances corrected for the analytical error. Table 4 shows the evaluation of the content uniformity of the suppositories according to both the fixed limit test of the USP XIX (1975) and to the calculation of the content precision. For all of the three drug substances the content uniformity complies with the USP claim and the content precisions were within 1.5%.

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REFERENCES

Bailey, F. and Brittain, P.N., High-efficiency liquid chromatography in pharmaceutical analysis. J. Chromatogr., 83 (1973) 431–437.

Cox, G.B., Loscombe, C.R., Slucutt, M.J., Sugden, K. and Upfield, J.A., The preparation, properties, and some applications of bonded ion-exchange packings based on microparticulate silicagel for high-performance liquid chromatography. J. Chromatogr., 117 (1976) 269–278.

Helboe, P. and Thomsen, M., High-performance liquid chromatographic determination of trimethoprim and sulphonamide combinations in pharmaceuticals. Arch. Pharm. Chem., Sci. Ed., 5 (1977) 25–32.

Helboe, P. and Thomsen, M., High-performance liquid chromatographic determination of mestranol and norethisterone in oral contraceptive tablets. Arch. Pharm. Chem., Sci., Ed., 6 (1978) 57–62.

Horvath, C., Melander, W., Molnar, I. and Molnar, P., Enhancement of retention by ion-pair formation in liquid chromatography with nonpolar stationary phases. Anal. Chem., 49 (1977) 2295–2305.

Jane, I., The separation of a wide range of drugs of abuse by high-pressure liquid chromatography. J. Chromatogr., 111 (1975) 227–233.

Knox, J.H. and Jurand, J., Separation of tetracyclines by high-speed liquid chromatography. J. Chromatogr., 110 (1975) 103–115.

Korpi, J., Wittmer, D.P., Sandemann, B.J. and Haney, W.G., Simultaneous analysis of hydrocortisone and hydrocortisone phosphate by high-pressure liquid chromatography: reversed-phase, ion-pairing approach. J. Pharm. Sci., 65 (1976) 1087–1089.

Lurie, I., Application of reversed phase ion-pair partition chromatography to drugs of forensic interest. J. Ass. Off. Anal. Chem., 60 (1977) 1035–1040.

Persson, B.-A. and Karger, B.L., High performance ion pair partition chromatography: the separation of biogenic amines and their metabolites. J. Chromatogr. Sci., 12 (1974) 521–528.

Sood, S.P., An Investigation of Factors Affecting the Reversed Phase Ion Pairing Approach to High Performance Liquid Chromatography. Diss., University of Missouri-Kansas City, Kansas City, 1976, pp. 17–19.

United States Pharmacopoeia, 19th. revision. New York, 1975, p. 648.

Wittmer, D.P., Nuessle, N.O. and Haney, W.G., Simultaneous analysis of tartrazine and its intermediates by reversed phase liquid chromatography. Anal. Chem., 47 (1975) 1422–1423.