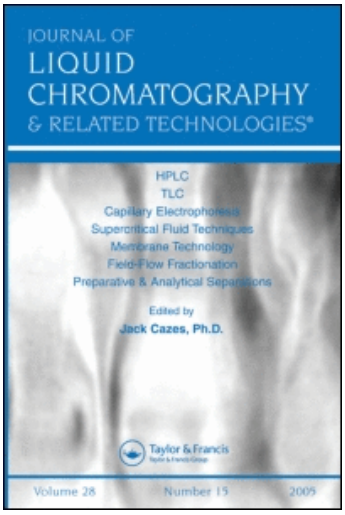

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Development of a Standardized Analysis Strategy for Basic Drugs, Using Ion-Pair Extraction and High-Performance Liquid Chromatography PART IV. Application to Solid Pharmaceutical Dosage Forms

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DEVELOPMENT OF A STANDARDIZED ANALYSIS STRATEGY FOR BASIC DRUGS, USING ION-PAIR EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

PART IV. Application to solid pharmaceutical dosage forms

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ABSTRACT

The usefulness of two standardized HPLC-systems for the analysis of basic drugs in tablets and capsules is exemplified. The standardized HPLC systems both use a CN-column combined with either a polar or a non polar mobile phase. Also the sample preparation is standardized and simple; it involves suspension of the powder mixture in one of the mobile phase components, centrifugation and injection of the clear supernatant.

INTRODUCTION

In previous papers from this laboratory (1-3) the development of a standardized analysis strategy for basic drugs was reported. This strategy combines an ion-pair extraction technique with two preferred HPLC-systems using a CN-bonded phase and either acetonitrile-water-propylamine (90:10:0.01) or n-hexane-dichloromethane-acetonitrile-propylamine (50:50:25:0,1) as standard mobile phases. The strategy is applicable to the determination of

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basic drugs in pharmaceutical dosage forms (syrups, ointments, emulsions...) (3), cosmetics (4), saliva and plasma (5). For solid pharmaceutical preparations such as tablets, the ion-pair extraction step can be omitted. The ground tablet can be suspended in the mobile phase component which is most appropriate for dissolving the analyte and following centrifugation the supernatant can directly be injected onto the nitrile bonded phase column. As reported earlier (3) the mobile phase composition can easily and rapidly be optimized for the particular analysis problem. The procedure is very convenient for the determination of basic drugs and is hence routinely applied in our laboratory in order to control label claims of tablets, capsules etc. The present paper describes some of these applications in order to exemplify the possibilities of the procedure. As in a previous paper (3) concerning the application of the strategy to syrups, ointments, etc. emphasis was laid on the extraction step and little or no account was given of how adaptation of the initial standardized mobile phase to the particular analysis problem was made, special attention will be paid in the present paper to the reasoning behind the alterations of the volume ratio of the mobile phase components.

EXPERIMENTAL

Apparatus

Chromatography was performed using either a Varian 5060 or a Varian 8500 liquid chromatograph, equipped with a Valco loop in-

jector (loop volume : 100 µl), a standard fixed wavelength (254 nm) UV detector, a Varian 9176 recorder and a Varian Vista CDS 401 data system. All analyses were performed using a LiChrosorb CN column, dp = 10 µm, 250 x 4 mm, except the caffeine analysis in which a Micro Pak CN-10 column, dp = 10 µm, 300 x 4 mm, was used. All analyses were performed at a flow rate of 2 ml/min and at maximum detector sensitivity in order to detect possible degradation products.

Chemicals and Reagents

n-hexane, dichloromethane and acetonitrile were HPLC grade and purchased from Fluka AG (Buchs, Switzerland) or E. Merck (Darmstadt, G.F.R.). Propylamine was obtained from Fluka AG (Buchs, Switzerland). Water was demineralized, double-distilled and further purified using a Water-I system (Gelman Sciences).

Composition of the Dosage Forms

Ponderal® Unicaps (Eutherapie Benelux, Belgium) : Fenfluramin. hydrochl. 60 mg - Hypromel. 50 - Cellul. - Saccharum. - Mononatrii phosphas. sic. - Talc - Magn. stear. - Eudragit S - Citroflex A_4 - Titan. oxyd. pro caps. gelat. una - Indigot. pro col. Titan. oxyd.

Vizocaf® coated tablets (Lab. Viselé, Belgium) : Diethylamid. Ac. Vanillic. 20 mg - Cafein. 20 mg - Amyl. - Sacch. lact. - Talc. - q.s. pro compressa - Accac. gum - Ti. oxy. - Talc. - Na. Indigotinodisulf. - Glycolpolyeth. - Sacch. pro obducta.

Rhinopront® capsules (Mack, G.F.R.) : Carbinoxamin. maleas 4 mg - Phenylephrin. hydrochlorid. 20 mg - Sacchar. - Maid. amyl. - Eudragit S mor. - Phtalic. acid. diaethylester - Talc. - Tartrazin. - Titan. dioxyd. - Natr. indigotinodisulfon. - Gelatin. pro capsula gelatinosa una.

Deanxit® coated tablets (Lundbeck, Denmark) : Flupentixol. dihydrochlorid. correspond. 0.5 mg basic. - Melitracen. hydrochlorid. correspond. 10 mg basic. - Amyl. - Sacchar. lact. - Gelatina - Talc. - Magnes. stearas pro tablet. compres. una - Indigotin. et Erythrosin. q.s. pro colore - Saccharo et Gelatina obducta.

Procedures

Determination of fenfluramine in Ponderal® Unicaps. The content of 20 capsules is pulverized in a mortar. An aliquot of the resulting powder, equivalent to 1/10 of the mean weight of a capsule is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml 0.001 M HCl. After ultrasonification for 10-15 min. the flask is brought to volume. After centrifugation, 100 µl of clear supernatant are injected onto the HPLC-column. Quantitation is effected by intrapolation on a calibration curve (peak area versus concentration) of five aqueous standards in the 8 - 16 µg fenfluramine HCl/100 µl range. The calibration curve is linear in this concentration range.

Determination of caffeine in Vizocaf® coated tablets. 20 coated tablets are pulverized in a mortar. An aliquot of the resulting powder, equivalent to 1/20 of the mean weight of a coated tablet is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml dichloromethane. After ultrasonication for 10 - 15 min the flask is brought to volume. After centrifugation, 100 µl of clear supernatant are injected onto the HPLC -column. Quantitation is effectuated by intrapolation on a calibration curve (peak area vs. concentration) of five standards in the 1 - 3 µg caffeine base/100 µl range, prepared in dichloromethane. The calibration curve is linear in this concentration range.

Determination of tetracaine in commercial tablets. 20 tablets are pulverized in a mortar. An aliquot of the resulting powder, equivalent to half the mean weight of a tablet is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml dichloromethane. After ultrasonication for 30 min the flask is brought to volume. After centrifugation, 100 µl of the clear supernatant are injected onto the HPLC-column. Quantitation is effected by intrapolation on a calibration curve (peak area vs. concentration) of five standards in the 0.3 - 0.7 µg/100 µl concentration range, prepared in dichloromethane. The calibration curve is linear in this concentration range.

Determination of carbinoxamine and phenylephrine in Rhinopront® capsules. All manipulations are effected as good as

possible protected from light. The content of 10 capsules is ground in a mortar. An aliquot of the resulting powder, equivalent to the mean weight of 1 capsule is accurately weighed, brought into a 100 ml volume flask and suspended in about 80 ml 0.001 M HCl. After ultrasonication for 10 - 15 min the flask is brought to volume. After centrifugation, 50 µl of clear supernatant are injected onto the HPLC-column. Quantitation is effected by intrapolation on calibration curves (peak area vs. concentration) of five aqueous standards containing from 2 to 6 mg carbinoxamine maleate/100 ml and from 15 to 25 mg phenylephrine hydrochloride/100 ml. Both calibration curves are linear in this concentration range.

Determination of flupentixol and melitracen in Deanxit® coated tablets. 25 coated tablets are pulverized in a mortar. An aliquot of the resulting powder, equivalent to the mean weight of one tablet is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml 0.001 M HCl. After ultrasonication for 10 - 15 min the flask is brought to volume. After centrifugation, 100 µl of clear supernatant is injected into the chromatograph. Quantitation is effected by intrapolation on calibration curves (peak area vs. concentration) of five aqueous standards containing from 0.5 to 1.5 µg flupentixol dihydrochloride/100 µl and from 15 to 25 µg melitracen hydrochloride/100 µl. Both calibration curves are linear in this concentration range.

RESULTS AND DISCUSSION

Fenfluramine in Ponderal®

The majority of the 100 basic drugs investigated previously (2) have capacity factors in the 1 - 10 range in both preferred eluents. Consequently both standardized eluents can be used for the chromatography of polar as well as non polar solutes. However, for fast optimization of the mobile phase composition for a drug not belonging to the original test set (2) we usually employ the polar mobile phase as initial investigation eluent for polar solutes and the non polar eluent for non polar molecules (3). Fenfluramine is a quite polar molecule and consequently the reversed phase system was used. In its original composition the acetonitrile-water-propylamine (90:10:0.01) eluent eluted fenfluramine with a retention time of 7.6 min at a flow rate of 2 ml/min. In order to shorten the analysis time the retention of the solute was reduced by increasing the propylamine content by a factor 3. A chromatogram of a capsule treated as described in the "Experimental section" is shown in Figure 1. As can be seen from Table 1, the recovery is excellent and the precision is acceptable.

Caffeine in Vizocaf®

The selection of an appropriate mobile phase for the determination of caffeine was very easy since its composition was identical to the eluent used for the determination of papaverine in blood (6). During method development for the latter assay the non

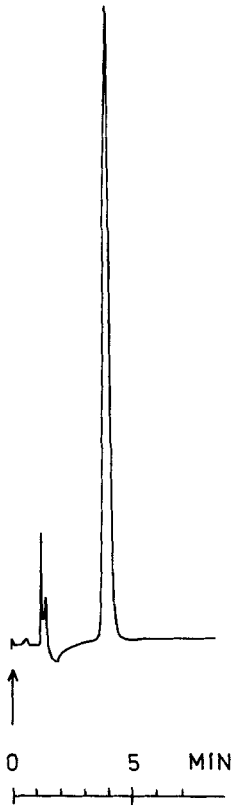

Figure 1 - Chromatogram of a Ponderal® capsule on a LiChrosorb-CN column (dp = 10 µm, 250 x 4 mm). Mobile phase : acetonitrile -water-propylamine (90:10:0.03). Flow rate : 2 ml/min. Detector sensitivity : 0.01 AUFS.

polar standardized eluent in its original composition n-hexane-dichloromethane-acetonitrile-propylamine (50:50:25:0.1) was altered by halving its dichloromethane content in order to resolve papaverine from caffeine and other matrix components. Since this (50:25:25:0.1) volume ratio allows caffeine to be chromatographed with good peak shape it was employed for the determination of caf-

TABLE 1

Chromatographic Conditions and Recoveries

Dosage Form	Column	Mobile Phase Composition		Analyte Retention time		Mean Recovery	(% of Label Claim)	± S.D.
Ponderal®	LiChrosorb-CN	Acetonitrile Water Propylamine	90 10 0.03	Fenfluramine	3.9	99.1	± 1.8%	(n=6)
Vizocaf®	MicroPak-CN	n-Hexane Dichloromethane Acetonitrile Propylamine	50 25 25 0.1	Caffeine	4.4	98.0	± 0.9%	(n=6)
Tetracaine tablets	LiChrosorb-CN	n-Hexane Dichloromethane Acetonitrile Propylamine	10 80 10 0.1	Tetracaine	3.0	64.9 (99.7	± 0.7% ± 0.9%)	(n=6) (n=3)
Rhino-pront®	LiChrosorb-CN	Acetonitrile Water Propylamine	80 20 0.05	Carbinoxamine Phenylephrine	2.7 5.2	97.9 100.2	± 0.7% ± 0.9%	(n=3)
Deanxit®	LiChrosorb-CN	Acetonitrile Water Propylamine	40 60 0.01	Flupentixol Melitracen	2.5 9.7	101.3 100.1	± 1.3% ± 1.8%	(n=6)

feine in Vizocaf® tablets. The column used was from the same manufacturer but from a different lot. It is striking to note that the retention time of caffeine (4.4 min) is much larger than on the column used for the papaverine determination from which caffeine eluted at 2.9 min. As stated previously (3) important differences between CN-columns of different batches and different brands indeed exist. This however does not jeopardize the standardized analysis strategy, since both preferred mobile phases are so versatile that the composition of the eluent can easily be finetuned in order to obtain retention times of a desired magnitude. Since in the case of the caffeine determination an analysis time of 5 min was considered sufficiently short, the mobile phase composition was not altered. A chromatogram of a tablet obtained as described in the "Experimental section" is shown in Figure 2. The recovery and standard deviation are excellent as can be seen from Table 1.

Tetracaine in Commercial Tablets

The determination was carried out because the tablets were suspected of being underdosed. The non polar standardized eluent in its original composition n-hexane-dichloromethane-acetonitrile -propylamine (50:50:25:0.1) resulted in strong retention of tetracaine. In order to shorten the time for analysing the tablet, the retention of tetracaine was reduced by drastically decreasing the hexane content of the mobile phase. The volume ratio of the mobile phase was then further finetuned to (10:80:10:0.1) in or-

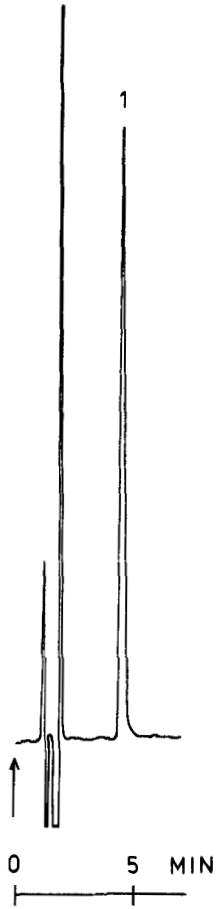

Figure 2 - Chromatogram of a Vizocaf® coated tablet on a Micro-Pak CN-10 column (dp = 10 µm, 300 x 4 mm). Mobile phase : n-hexane-dichloromethane-acetonitrile-propylamine (50:25:25:0.1). Flow rate : 2 ml/min. Detector sensitivity : 0.08 AUFS.

der to obtain a good peak shape. A chromatogram of a tablet treated as described in the "Experimental section" is given in Figure 3a. As can be seen from Table 1, the tablet contains only 64.9% of the tetracaine label claim which confirms the suspicion of underdosing. The precision of the method is excellent. In order to evaluate the accuracy of the method, a self made powder-mixture containing all ingredients of the commercially available tablet was analysed. The recovery of tetracaine was 99.7% $\pm$ 0.9% (n = 3) which confirms the accuracy of the method. The chromatograms of the commercial tablets showed only one peak (tetracaine) suggesting that no degradation of tetracaine had taken place. The main degradation product of tetracaine is p-n-butylaminobenzoic acid (7). Since we did not know whether this degradation product would be detectable with the chromatographic conditions used it was decided to proceed as follows. An aqueous tetracaine solution was subjected to hydrolysis at alkaline pH and at an elevated temperature ($\pm$ 90°C). An aliquot of this solution was extracted with dichloromethane after acidification. The extract was subjected to HPLC-analysis and in the chromatogram no peak at the retention time of tetracaine (t_R = 3.0 min) was observed but instead a peak with t_R = 1.7 min emerged. Consequently this peak was tentatively identified as p-n-butylaminobenzoic acid. The absence of such a peak in the chromatograms of the commercial tablets suggests that not hydrolysis but underdosing is responsible for the low tetracaine recovery. A chromatogram of both tetracaine and its degradation product, obtained by injecting a mixture

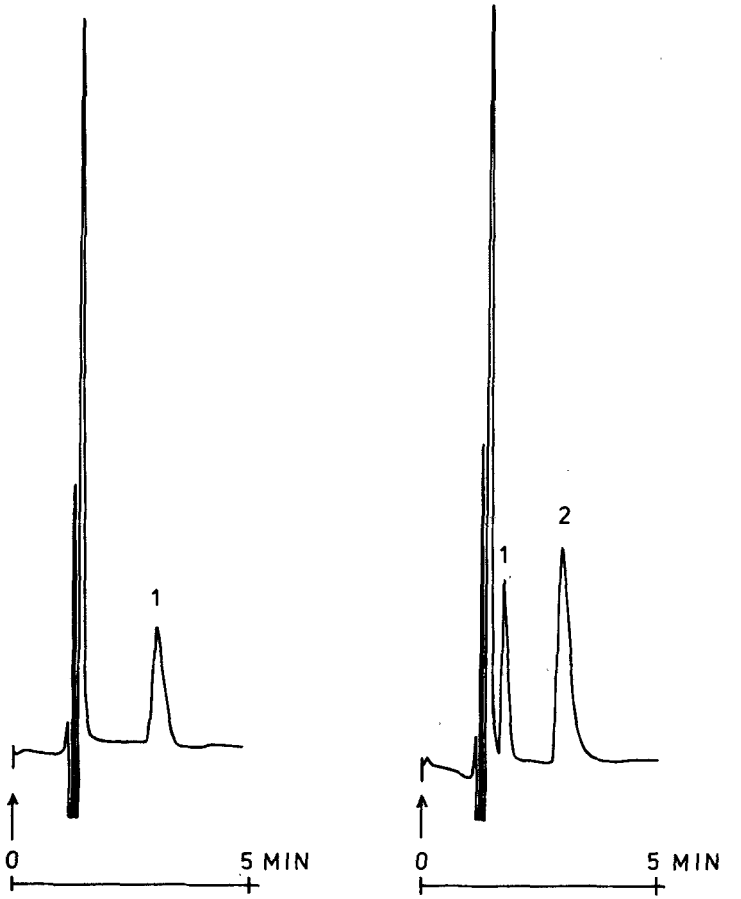

Figure 3 - a) Chromatogram of a commercial tetracaine tablet (Peak 1 = tetracaine) and; b) Chromatogram of a mixture of Tetracaine (= Peak 2) and its main degradation product (= Peak 1) on a LiChrosorb-CN column (dp = 10 µm, 250 x 4 mm). Mobile phase : n-hexane-dichloromethane-acetonitrile-propylamine (10:80:10:0.1). Flow rate : 2 ml/min. Detector sensitivity : 0.01 AUFS.

of the extract of the hydrolytic solution and a tetracaine standard is shown in Figure 3 b.

Other local anesthetics have also been chromatographed using eluents emanating from the nonpolar standardized mobile phase and the eluent has also been optimized for the separation of seven local anesthetics (8). A chromatogram of this separation using a (50:75:20:0.1) volume ratio is shown in Figure 4.

Carbinoxamine and Phenylephrine in Rhinopront®

The present application is reported in order to demonstrate the suitability of the CN-column for the simultaneous determination of a polar (phenylephrine) and a nonpolar (carbinoxamine) solute in a single chromatographic run with a fairly short analysis time. Usually this is difficult to achieve either on a C_{18}- or on a Si-column. On a Si-column it is indeed usually difficult to obtain sufficient retention of a nonpolar solute while avoiding excessive retention of the polar molecule. Similar difficulties occur with a C_{18}-column unless ion-pair chromatography would be used. The use of a CN-column which is of intermediate polarity offers however a more convenient solution. Optimization of the acetonitrile-water-propylamine (90:10:0.01) eluent was performed as follows. In its original composition this eluent resulted in a considerable retention time for phenylephrine and a rather unfavourable peak shape. Improvement could be obtained by increasing the water content of the eluent to 50-60% but this had a negative effect on the selectivity. It was therefore decided to increase

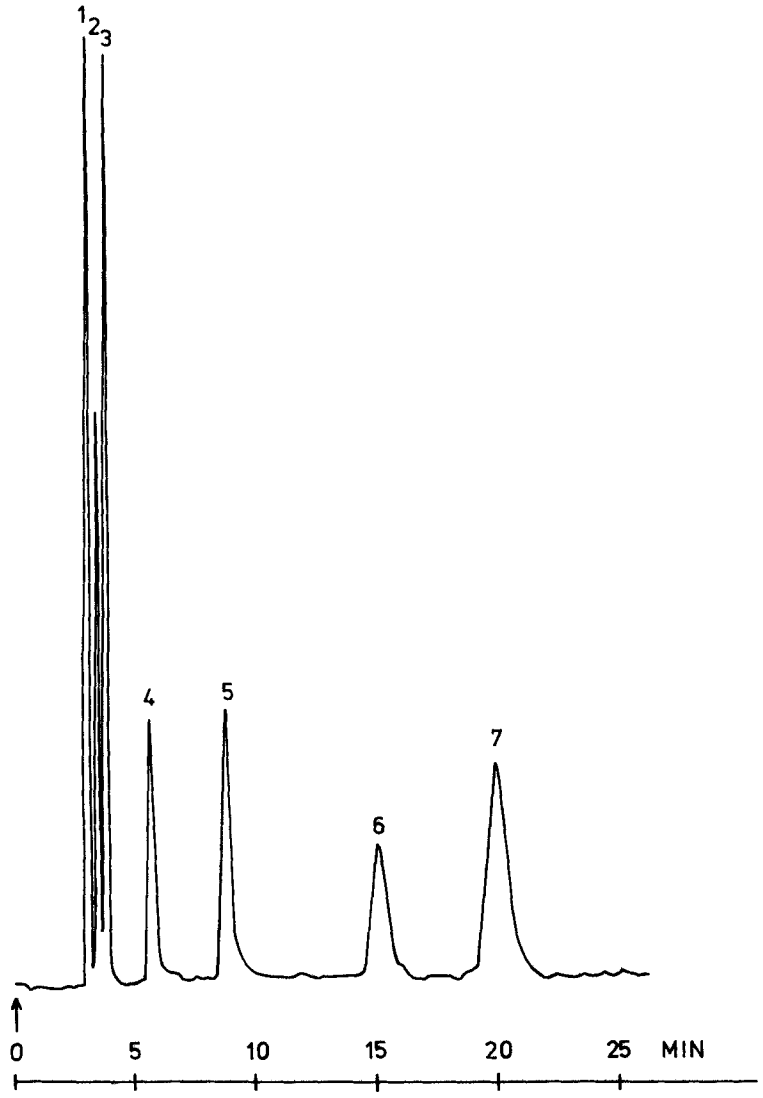

Figure 4 - Chromatogram of a mixture of local anesthetics on a MicroPak CN-10 column (dp = 10 µm; 300 x 4 mm). Peak identification : 1. Amylocaine, 2. Lidocaine, 3. Benzocaine, 4. Mepivacaine, 5. Piperocaine, 6. Procaine, 7. Tetracaine. Mobile phase : n-hexane-dichloromethane-acetonitrile-propylamine (50:75:20:0.1). Flow rate : 1 ml/min. Detector sensitivity : 0.01 AUFS.

the propylamine content by a factor 5 while increasing the water content to only 20%. The optimum volume ratio is hence : (80:20:0.05). A chromatogram (Figure 5) of a Rhinopront capsule treated as described in the "Experimental section" shows that both the polar and the nonpolar drug can be quantified simultaneously while limiting the analysis time to 7 min. The recoveries and standard deviations are also excellent as can be seen from Table 1.

Flupentixol and melitracen in Deanxit®

In the two foregoing examples in which the polar standardized mobile phase was used, the composition of the eluent had to be altered in order to reduce the retention of the polar analytes. If one wishes to chromatograph nonpolar solutes using the acetonitrile-water-propylamine eluent it sometimes is necessary to enhance retention. In the same way as for a classical reversed phase system using a C_{18}-column this usually can be done by increasing the water content of the mobile phase. The present application is reported to exemplify this. Flupentixol and melitracen are rather poorly retained when the original acetonitrile -water-propylamine (90:10:0.01) eluent is used. Drastically increasing the polarity of the mobile phase to a (50:50:0.01) volume ratio resulted in appropriate retention as can be seen in Figure 6. However, at this mobile phase composition flupentixol was not entirely separated from a tablet ingredient (probably one of the dyes). A baseline separation of flupentixol and the interfering sample ingredient could be achieved by further increasing the

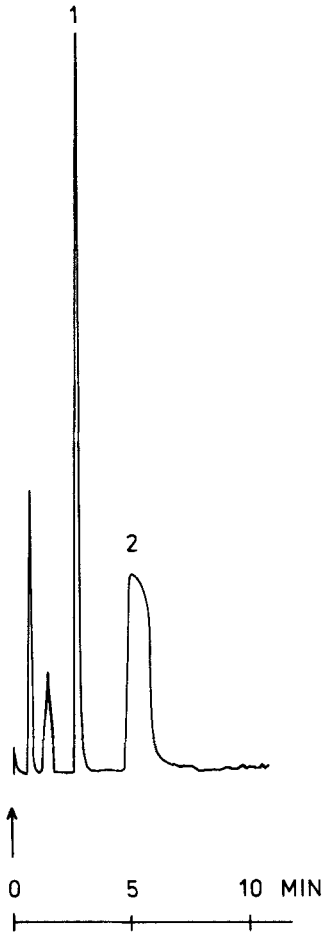

Figure 5 - Chromatogram of a Rhinopront® capsule on a LiChrosorb -CN column (dp = 10 µm, 250 x 4 mm). Peak identification : 1. carbinoxamine, 2. phenylephrine. Mobile phase : acetonitrile -water-propylamine (80:20:0.05). Flow rate : 2 ml/min. Detector sensitivity : 0.08 AUFS.

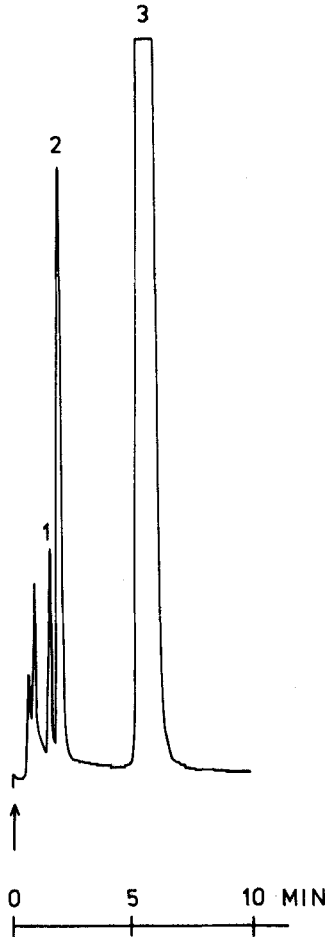

Figure 6 - Chromatogram of a Deanxit® coated tablet on a LiChrosorb-CN column (dp = 10 µm, 250 x 4 mm). Peak identification : 1. tablet ingredient, 2. flupentixol, 3. melitracen. Mobile phase : acetonitrile-water-propylamine (50:50:0.01). Flow rate : 2 ml/min. Detector sensitivity : 0.02 AUFS.

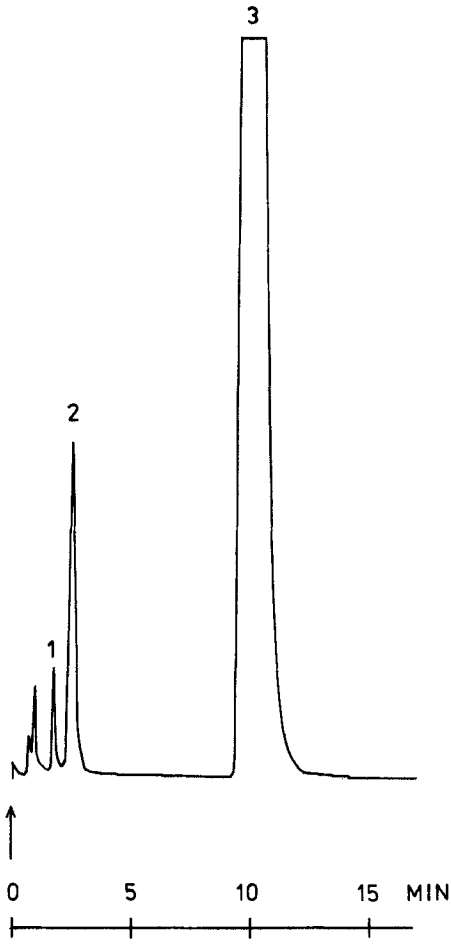

Figure 7 - Chromatogram of a Deanxit® coated tablet. Mobile phase : acetonitrile-water-propylamine (40:60: 0.01). All other parameters are the same as in Figure 6.

polarity of the eluent, at the cost however of also increasing the analysis time. A chromatogram of a Deanxit® coated tablet using a volume ratio of (40:60:0.01) is shown in Figure 7. The recoveries and standard deviations, presented in Table 1, are acceptable.

CONCLUSIONS

A few applications have been reported in order to exemplify the suitability of the standardized HPLC-systems for the determination of basic drugs in solid pharmaceutical dosage forms. The advantages of the approach are the following :

1) column selection can be omitted since all assays are performed using a CN-column
2) both polar and nonpolar solutes can be chromatographed on this column, and as was demonstrated, simultaneously if needed
3) both standardized mobile phases are very versatile and are easily and rapidly adaptable to the particular analysis problem
4) the excellent separation ability of the standardized HPLC-systems, even for stereoisomers (e.g. ref. 3,4) is profitable for stability indicating tests as was shown previously (3) and presently in the tetracaine case
5) the recovery and precision data show that the use of an internal standard is not necessary.

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