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Development of a standardized analysis strategy for basic drugs, using ion-pair extraction and high-performance liquid chromatography — III. Analysis of pharmaceutical dosage forms*

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Abstract: The usefulness of the standardized analysis strategy previously described for the determination of basic drugs in pharmaceutical dosage forms is evaluated. Several examples which typify experience in applying the strategy are reported. Ion-pair extraction techniques are compared with each other and with a classical extraction method in terms of their efficiency. The extraction technique with di(2-ethylhexyl)-phosphoric acid (HDEHP) is found to be the method of choice. The use of an internal standard is recommended. The selection of a suitable compound is greatly facilitated by referring to the chromatographic properties. It is shown that it is not necessary for the analyte and internal standard to be structurally similar. The combination of the HDEHP extraction technique with the preferred HPLC systems has been shown to be very useful in the routine analysis of pharmaceutical dosage forms.

Keywords: *Standardized analysis strategy; basic drugs; ion-pair extraction; pharmaceutical dosage forms; stability-indicating assay.*

Introduction

In part I of this series of articles on the development of a standardized analysis strategy for basic drugs, the philosophy of the strategy was explained [1]. It was also shown that an ion-pair extraction technique with either sodium *n*-octylsulphate (NaOS) or di(2-ethylhexyl)-phosphoric acid (HDEHP), combined with direct injection of the extracts on to an HPLC system chosen from two preferred HPLC systems [2], might be useful for the determination of basic drugs in various matrices [1]. In the present paper the usefulness of the strategy for the determination of basic drugs in pharmaceutical dosage forms is evaluated by the analysis of a number of syrups, emulsions, ointments and other preparations. Most of the pharmaceutical preparations investigated were analysed in fulfilment of a governmental control assignment; others (e.g. ephedrine ointment) were selected in order to evaluate the strategy as applied to very hydrophilic compounds. The

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examples and results reported in the present paper are typical of the authors' experience in applying the strategy in pharmaceutical analysis.

Experimental

Apparatus

A Varian 5060 liquid chromatograph was used, equipped with a Valco loop-valve injector (loop volume 100 μl), a fixed wavelength UV detector (254 nm), a Varian 9176 recorder and a Varian Vista CDS 401 chromatographic data system. All analyses were performed with a 250 × 4 mm i.d. LiChrosorb-CN column (d_p = 10 μm).

Chemicals and reagents

Sodium-*n*-octylsulphate (for tenside tests) was purchased from E. Merck (Darmstadt, FRG). Di(2-ethylhexyl) phosphoric acid from Koch-Light Laboratories Ltd. (Colnbrook, Bucks, England) was purified as described previously [1]. All drugs were of pharmacopoeial or equivalent purity. *n*-Hexane, dichloromethane and acetonitrile were HPLC grade and purchased either from Fluka AG (Buchs, Switzerland) or E. Merck (Darmstadt, FRG). All other reagents were analytical-reagent grade and obtained from E. Merck (Darmstadt, FRG), except for propylamine which was purchased from Fluka AG (Buchs, Switzerland).

Composition of the pharmaceutical dosage forms

Zaditen® Syrup (Wander Sandoz, Switzerland): ketotifen 20 mg (as hydrogen fumarate 27.6 mg), synthetic aromatic flavour (derog. 42/500), propyl *p*-hydroxybenzoate, methyl *p*-hydroxybenzoate, anhydrous citric acid, anhydrous disodium hydrogen phosphate, ethanol, sucrose, sorbitol and purified water to 100 ml.

Doxergan® Syrup (Rhône-Poulenc, France): oxomemazin 5 mg, citric acid, sucrose, saccharin sodium, flavours and colours (derog. 41/33 and 42/45) and water to 5 ml.

Diphenhydramini Emulsio® (Vandenbussche, Belgium): diphenhydramine hydrochloride 10 mg, liquid wax, liquid paraffin, geranium oil, sorbitol tristearate, sorbitol monostearate and water to 1 ml (0.97 g). The propellant was nitrogen.

Ephedronguent® Ointment (Pharmacobel, Belgium): ephedrine hydrochloride 333.3 mg, boric acid (trace), almond oil, wool fat, geranium oil and soft paraffin to 10 g.

Polaramine® Syrup (Schering, USA): chlorpheniramine maleate 2 mg, sodium chloride, sodium citrate, sucrose, sorbitol, synthetic apricot essence, ethanol, menthol, synthetic orange essence, propylene glycol, methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate and purified water to 4 ml.

Primperan® Suppositories (Delagrange, France): metoclopramide base 10 mg in a glyceride basis.

Extraction procedures

All extractions were carried out in centrifuge tubes equipped with PTFE-covered screw-caps. Partitioning was performed by gently shaking the tubes longitudinally in a

shaking bath for 30 min. After centrifugation the phases were separated and 100 μl of the extract was injected on to the column.

Zaditen Syrup. To 1 ml syrup or 1 ml aqueous solution containing 276 μg ketotifen hydrogen fumarate/ml (= 200 μg ketotifen base/ml) were added:

(a) 9 ml NaOS (0.05 M) in phosphate buffer pH 3.0 (ionic strength, $\mu = 0.4$) and 10 ml chloroform; or

(b) 9 ml phosphate buffer pH 5.5 ($\mu = 0.4$) and 10 ml HDEHP (0.001 M) in chloroform; or

(c) 9 ml phosphate buffer pH 10.0 ($\mu = 0.4$) and 10 ml chloroform.

The recoveries were determined by interpolation from a calibration curve (peak area against concentration) of five standards in the concentration range 10–30 μg/ml, prepared in acetonitrile.

Doxergan® Syrup. To 1 ml syrup and 1 ml internal standard solution (100 μg bamipine lactate/ml water) were added:

(a) 8 ml NaOS solution (0.05 M) and 10 ml chloroform; or

(b) 8 ml buffer pH 5.5 and 10 ml HDEHP (0.001 M) in chloroform; or

(c) 8 ml buffer pH 10.0 and 10 ml chloroform.

The recoveries were determined by interpolation from two linear calibration curves [peak area against concentration and peak area ratio of oxomemazine: internal standard (IS) against concentration] of five standards in the concentration range 10–50 μg/ml, prepared in chloroform.

Diphenhydramini Emulsio®. To 0.5000 g emulsion and 1 ml IS solution (oxomemazine 1 mg/ml in water) was added 9 ml of the respective buffer solution. After thorough shaking, the mixture was subjected to ultrasonication for 15 min and then filtered. Five ml of the filtrate were then extracted with 5 ml of the corresponding organic phase.

The recoveries were determined by interpolation from two calibration curves (peak area against concentration and peak area ratio of diphenhydramine: IS against concentration) of five standards in the concentration range 100–800 μg/ml, each containing 100 μg/ml oxomemazine, prepared in chloroform.

Ephedronguent®. To 0.1000 g ointment and 1 ml IS solution (mephentermine HCl 3 mg/ml in water) was added 9 ml of the respective buffer solution. The tubes were shaken in a water-bath at 75°C for 30 min and after cooling the contents were filtered. Five ml of the filtrate was then extracted with 5 ml of the corresponding organic phase. In this case the HDEHP concentration was 0.01 M instead of 0.001 M.

The recoveries were determined by interpolation from two calibration curves (peak area against concentration and peak area ratio of ephedrine: IS against concentration) of four standards in the concentration range 100–350 μ/ml, each containing 300 μg mephentermine HCl/ml prepared in water.

Polaramine®. To 1 ml syrup and 1 ml IS solution (quinine 0.6 mg/ml in water) were added 8 ml of the respective buffer solution and 10 ml of the respective organic phase.

The recoveries were determined by interpolation from two calibration curves of five standards in the concentration range 10–80 μg/ml, each containing quinine HCl 600 μg/ml in chloroform.

Primperan®. An entire suppository, 10 ml IS solution (tiapride HCl 1.01 mg/ml in water) and 90 ml HCl (0.01 M) were mixed in a closed vessel which was heated at 40°C in a water-bath for 5 min. After homogenization, the emulsion was allowed to cool and then filtered. To 1 ml of the filtrate was added 9 ml of the respective buffer solutions and 10 ml of the respective organic phases.

The recoveries were determined by interpolation from two calibration curves of five standards in the range 5–15 μg/ml, each containing tiapride HCl 10.1 μg/ml in chloroform.

Results and Discussion

Extraction

The efficiencies of both ion-pair extraction techniques were compared with each other and with classical extraction at pH 10.0. In all experiments chloroform was used as the solvent. The NaOS extraction technique was performed at pH 3.0 with a 0.05 M NaOS, whereas the HDEHP extraction technique was performed at pH 5.5 with 0.001 M HDEHP (0.01 M HDEHP for the ephedrine ointment). In all experiments an aqueous phase–organic phase ratio of 1:1 (v/v) was used.

Chromatography

One of the two preferred mobile phases [2] was optimized for each particular problem. The initial choice of whether to use the polar or the apolar eluent was based on the polarity of the analyte. For very polar compounds, the polar eluent was best suited whereas for less polar compounds, the apolar mobile phase was used as the eluent in initial investigations. Optimization of the mobile phase composition was carried out in order to:

(1) obtain a symmetrical shape for the analyte peak; (2) allow separation of the analyte from excipients and degradation products which could be expected to be co-extracted; and (3) reduce the analysis time.

Practical experience with the preferred HPLC systems and the use of a three-solvent delivery system resulted in an extremely fast optimization of the mobile phase composition. The final chromatographic conditions are presented in Table 1.

Determination of ketotifen in Zaditen® Syrup

Typical chromatograms are shown in Fig. 1 and the total analytical recoveries are presented in Table 2. It can be seen that none of the three extraction methods allowed fully quantitative recovery of ketotifen from the syrup. Since this might also be the case in the analyses to follow, it was decided to examine the use of the internal standard technique in order to compensate for drug loss during the extraction procedure.

Determination of oxomemazine Doxergan® Syrup

Typical chromatograms are shown in Fig. 2 and the recoveries are presented in Table 3. The recoveries obtained by use of the analyte peak area show that only the HDEHP extraction technique allowed quantitative recovery.

The use of an internal standard (IS) allows compensation to be made for loss of analyte during the extraction procedure, provided it is extracted with the same efficiency as the analyte. As IS it is customary to choose a substance that is chemically similar to that of interest, although chemical similarity does not always ensure similar extraction

Table 1
Chromatographic conditions*

Pharmaceutical dosage form	Mobile phase composition (ratio, v/v)		Flow rate (ml/min)	Detector sensitivity (a.u.f.s.)†	Retention time of analyte (min)	Retention time of internal standard (min)	
Zaditen®	Hexane Dichloromethane Acetonitrile Propylamine	70 15 15 0.1	4	0.04	1.7	—	
Doxergan®	Hexane Dichloromethane Acetonitrile Propylamine	80 10 10 0.1	4	0.08	2.4	Bamipine	1.8
Diphenhydramini Emulsio®	Hexane Dichloromethane Acetonitrile Propylamine	80 10 10 0.1	4	0.08	1.6	Oxomemazine	2.4
Ephedronguent®	Acetonitrile Water Propylamine	80 20 0.1	2	0.04	4.5	Mephentermine	5.5
Polaramine®	Hexane Dichloromethane Acetonitrile Propylamine	30 50 20 0.1	2	0.08	3.4	Quinine	5.4
Primperan®	Hexane Dichloromethane Acetonitrile Propylamine	10 10 80 0.1	2	0.04	4.1	Tiapride	2.3
Ben-U-Ron®	Hexane Dichloromethane Acetonitrile Propylamine	30 50 20 0.1	2	0.16	3.7	—	
Cafergot®-PB	Hexane Dichloromethane Acetonitrile Propylamine	70 15 15 0.1	2	0.04	6.0	—	

* Based on a 250 × 4 mm i.d. LiChrosorb-CN column (d_p = 10 μm).
† a.u.f.s. = absorbance units for full-scale deflection.

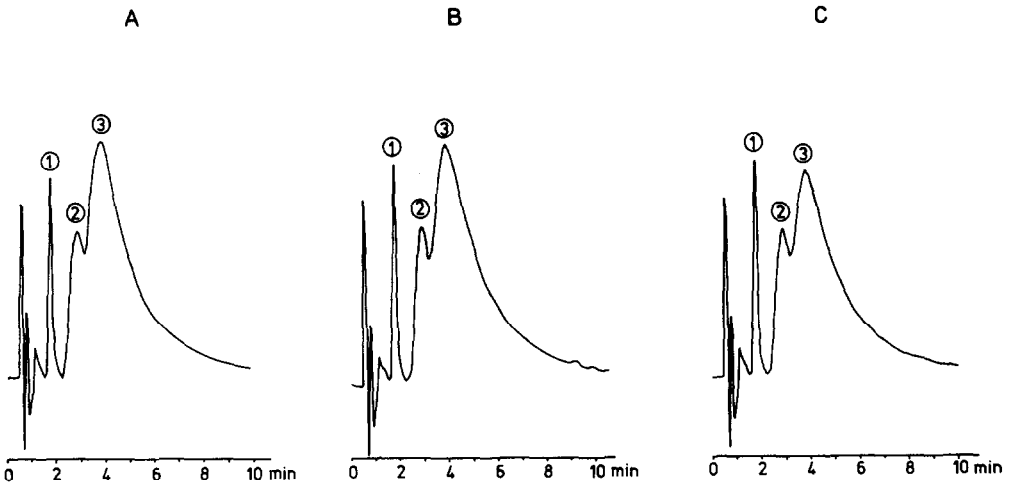

Figure 1
Chromatograms of a NaOS extract (A), an HDEHP extract (B) and a classical extract (C) of Zaditen® Syrup on a LiChrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, ketotifen; 2, propyl *p*-hydroxybenzoate; 3, methyl *p*-hydroxybenzoate.

Table 2
Recoveries of ketotifen by the three extraction techniques*

Sample	Extraction technique		
	NaOS†	HDEHP‡	Classical§
Aqueous solution (n=3)	97.2 ± 1.6	102.1 ± 0.7	103.8 ± 1.8
Zaditen® Syrup (n=6)	88.9 ± 1.9	97.5 ± 1.1	98.5 ± 1.5

* Recoveries are expressed as a percentage of the theoretical amount (±S.D.).
† Sodium *n*-octylsulphate (0.05M) in pH 3.0 phosphate buffer (μ = 0.4)–chloroform.
‡ Phosphate buffer (pH 5.5; μ = 0.4)–0.001 M di(2-ethylhexyl)-phosphoric acid in chloroform.
§ Phosphate buffer (pH 10.0; μ = 0.4)–chloroform.

behaviour [3, 4]. Nevertheless, the IS should be sufficiently different from the analyte to allow complete resolution under the given chromatographic conditions. These requirements often make the selection of an IS a difficult task [5] that becomes particularly time-consuming when the IS has to be synthesized, when the chromatographic conditions have to be altered in order to allow separation of the selected IS from the sample components, or when the extraction procedure has to be altered in order to obtain similar recoveries for both IS and analyte.

One of the advantages of the ion-pair extraction technique with NaOS or HDEHP as the ion-pairing reagent is in the comparably good extraction yields for drugs, even for those with very different structures [1]. The choice of IS is consequently made much easier, since there is no need for structural similarity of the IS. It suffices to choose a substance that undergoes ion-pair extraction, is eluted near the analyte peak and is well resolved from all components of the sample.

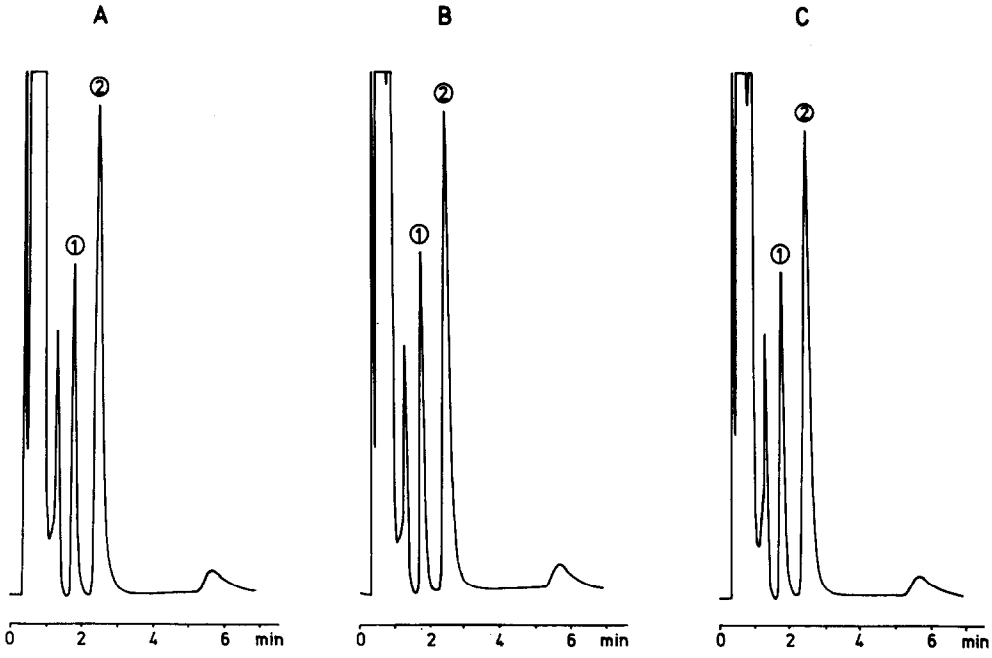

Figure 2
Chromatograms of a NaOS extract (A), an HDEHP extract (B) and a classical extract (C) of Doxergan® Syrup on a LiChrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, bamipine, internal standard (IS); 2, oxomemazine.

Table 3
Recoveries of oxomemazine from Doxergan® Syrup using three extraction techniques*

Calibration method	Correlation coefficient for calibration graph	Extraction technique† NaOS	HDEHP	Classical
Peak area	$r = 0.9998$	93.9 ± 2.0	100.5 ± 0.3	91.7 ± 4.7
Peak area ratio to bamipine (IS)	$r = 0.9997$	100.3 ± 1.5	100.5 ± 0.3	96.0 ± 2.6

* Recoveries are expressed as a percentage of the label claim (±S.D).
† $n = 6$; for details see text.

For the determination of oxomemazine in Doxergan® Syrup, the choice of the IS was made by injecting five, randomly chosen antihistamine drugs (bromazine, alimemazine, bamipine, carbinoxamine and diphenylpyraline) into the chromatographic system as optimized for oxomemazine.

Bamipine was selected as IS because of its good peak symmetry and its elution near the oxomemazine peak, thus demonstrating that the choice of IS can be made very quickly.

The recoveries for oxomemazine using the peak area ratio of oxomemazine: IS as the quantitative criterion are also presented in Table 3. The following conclusions can be drawn:

(1) Both quantitative criteria yield the same recovery and S.D. for the HDEHP extraction technique, showing that the extraction of both oxomemazine and of bamipine is quantitative and reproducible.

(2) Using the IS technique a similar recovery for the NaOS technique is obtained as for the HDEHP technique.

(3) The classical extraction technique results in lower recoveries than either of the ion-pair extraction techniques, irrespective of the quantitative criterion applied. It also appears that the use of bamipine as IS does not allow correction for loss of analyte during classical extraction. It can thus be deduced that the extraction recoveries of oxomemazine and bamipine are not similar under these conditions. Finally, it should be noted that the classical extraction technique results in a somewhat higher S.D. compared to either of the ion-pairing techniques.

It can thus be concluded, at least for this particular example, when using either of the ion-pair extraction techniques there is no need for chemical similarity of analyte and IS. It can further be stated that the ion-pair extraction techniques are both suitable for the determination of oxomemazine in Doxergan® Syrup; they are to be preferred to the classical extraction technique. For the NaOS extraction method, the use of an IS is necessary.

Determination of diphenhydramine in Diphenhydramini Emulsio®

Typical chromatograms are shown in Fig. 3 and the recoveries are presented in Table 4. It can be seen that the extraction of diphenhydramine from the emulsion is not quantitative, no matter which extraction method is used. However, the yields obtained

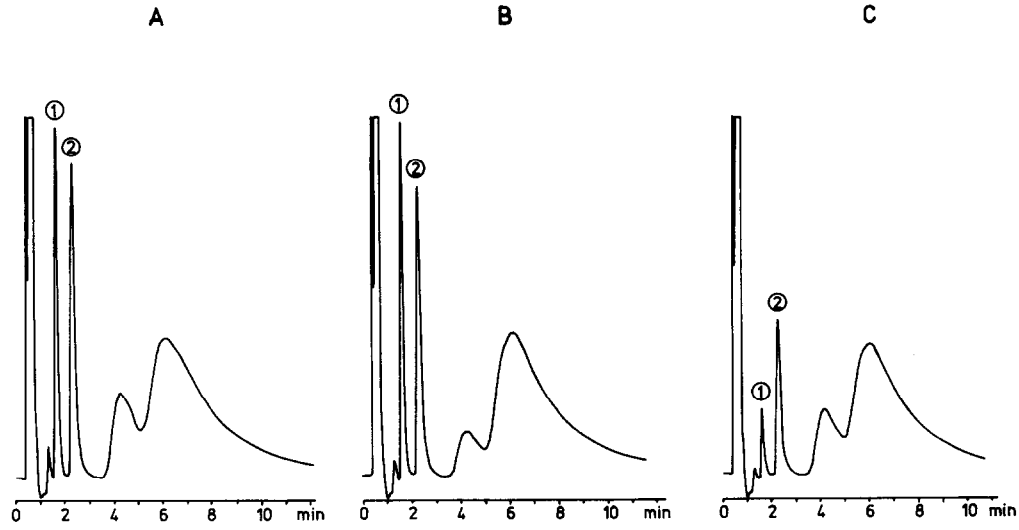

Figure 3
Chromatograms of a NaOS extract (A), an HDEHP extract (B) and a classical extract (C) of Diphenhydramini Emulsio® on a LiChrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, diphenhydramine; 2, oxomemazine (IS).

Table 4
Recoveries of diphenhydramine from Diphenhydramini Emulsio® using three extraction techniques*

Calibration method	Extraction technique†			
	Correlation coefficient for calibration graph	NaOS	HDEHP	Classical
Peak area	$r = 1.0000$	93.1 ± 1.2	92.6 ± 1.5	18.7 ± 0.7
Peak area ratio to oxomemazine (IS)	$r = 0.9999$	99.6 ± 0.7	102.9 ± 0.9	38.9 ± 0.1

* Recoveries are expressed as a percentage of the label claim (±S.D).
† $n = 6$; for details see text.

with both ion-pairing techniques are similar and much higher than the recovery for the classical extraction method.

Table 4 also presents the results obtained when using oxomemazine as the internal standard, selected according to the criteria discussed above. The IS technique allows quantitative yields to be obtained for both ion-pair extraction techniques, but not for the classical extraction method. It can thus be concluded that by use of the internal standard technique, both ion-pair extraction techniques can be applied to the determination of diphenhydramine in Diphenhydramini Emulsio® and are to be preferred to the classical extraction method. In this example it is also demonstrated that structural similarity of analyte and IS is not necessary, provided that one of the ion-pair extraction techniques is employed.

Determination of ephedrine in Ephedronguent® Ointment

Typical chromatograms are shown in Fig. 4 and the recoveries are presented in Table 5. It can be seen that only the HDEHP extraction technique results in acceptable recoveries. The low recovery with the NaOS technique is almost certainly due to an unfavourable counter-ion:analyte concentration ratio. However, the concentration of the NaOS solution could not be drastically increased without exceeding the critical micellar concentration.

On the other hand, decreasing the ephedrine concentration might result in sensitivity problems. The use of an internal standard (mephentermine) only yielded quantitative results for the HDEHP extraction technique. The conclusion that HDEHP is the more generally applicable ion-pairing reagent since it yields superior extraction recoveries for very hydrophilic drugs [1] is consequently confirmed.

Determination of chlorpheniramine in Polaramine® Syrup

Typical chromatograms are shown in Fig. 5 and the recoveries are presented in Table 6. As in the previous example a rather low recovery is obtained using the octylsulphate extraction technique, due to an unfavourable counter-ion:analyte concentration ratio. The HDEHP extraction technique results in higher yields, which are also superior to those obtained with the classical extraction technique. Once again the HDEHP

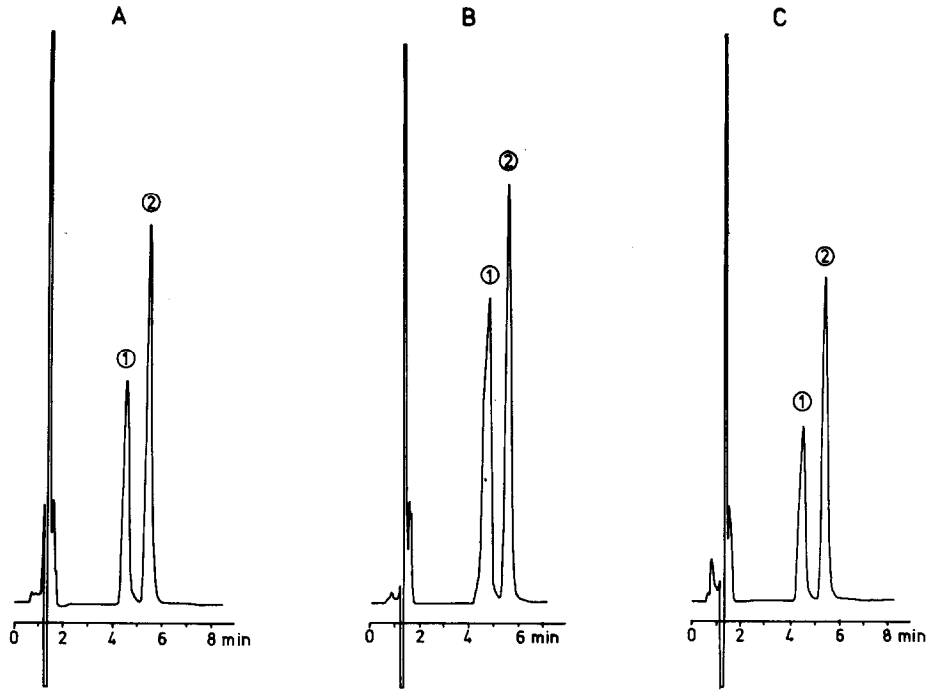

Figure 4
Chromatograms of an NaOS extract (A), an HDEHP extract (B) and a classical extract (C) of Ephedronguent® on a LiChrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, ephedrine; 2, mephentermine (IS).

Table 5
Recoveries of ephedrine from Ephedronguent® using three extraction techniques*

	Extraction technique†			
Calibration method	Correlation coefficient for calibration graph	NaOS	HDEHP	Classical
Peak area	$r = 0.9990$	50.7 ± 3.9	96.2 ± 2.8	43.4 ± 2.9
Peak area ratio to mephentermine (IS)	$r = 0.9995$	56.6 ± 3.1	99.7 ± 2.1	56.8 ± 2.8

* Recoveries are expressed as a percentage of the label claim (±S.D).
† $n = 6$; for details see text.

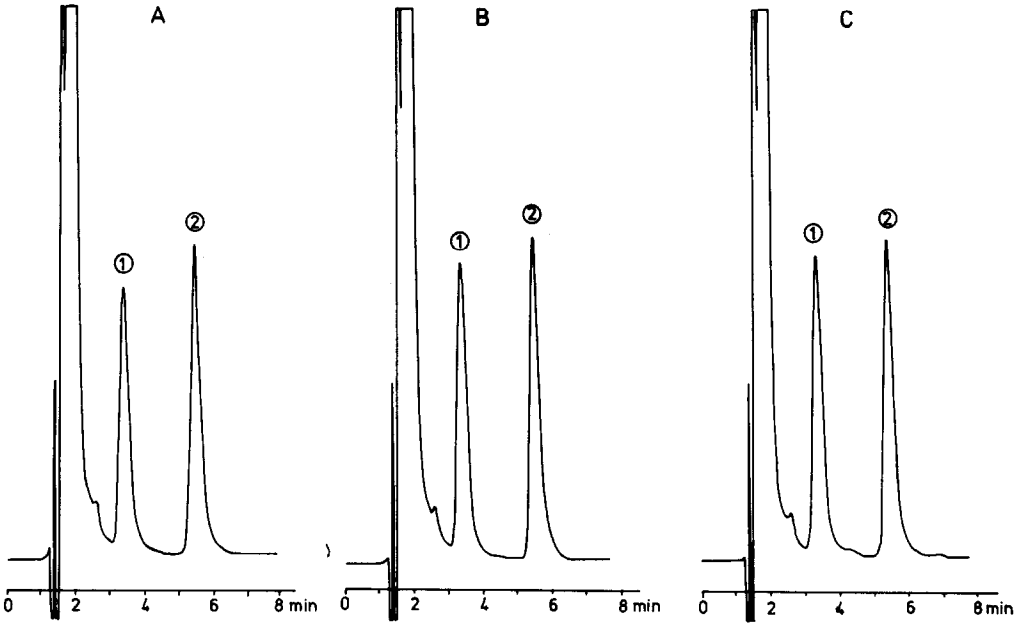

Figure 5
Chromatograms of an NaOS extract (A), an HDEHP extract (B) and a classical extract (C) of Polaramine® Syrup on a LiChrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, chlorpheniramine; 2, quinine (IS).

Table 6
Recoveries of chlorpheniramine from Polaramine® Syrup using three extraction techniques*

Calibration method	Extraction technique†			
	Correlation coefficient for calibration graph	NaOS	HDEHP	Classical
Peak area	$r = 0.9979$	85.4 ± 2.7	97.0 ± 3.3	95.9 ± 2.9
Peak area ratio to quinine (IS)	$r = 0.9988$	88.3 ± 1.7	100.0 ± 1.0	98.5 ± 1.4

* Recoveries are expressed as a percentage of the label claim (±S.D).
† $n = 6$; for details see text.

technique is the preferred extraction method and once again it is shown that structural similarity between analyte and internal standard is not required.

Determination of metoclopramide in Primperan® Suppositories

Typical chromatograms are shown in Fig. 6 and the recovery results are presented in Table 7. Again the HDEHP extraction technique turns out to be the method of choice, with the use of an internal standard (tiapride).

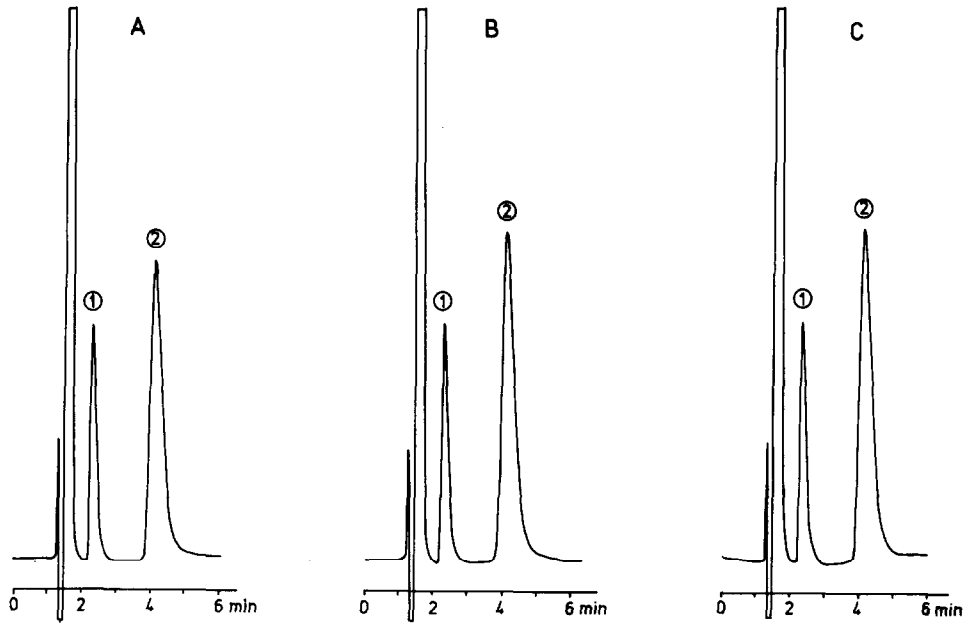

Figure 6
Chromatograms of an NaOS extract (A), an HDEHP extract (B) and a classical extract (C) of a Primperan® Suppository on a LiChrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, tiapride (IS); 2, metoclopramide.

Table 7
Recoveries of metoclopramide from Primperan® Suppositories using three extraction techniques*

Calibration method	Extraction technique†			
	Correlation coefficient for calibration graph	NaOS	HDEHP	Classical
Peak area	$r = 0.9988$	74.2 ± 2.5	86.3 ± 1.9	84.3 ± 1.5
Peak area ratio to tiapride (IS)	$r = 0.9999$	90.3 ± 2.0	99.7 ± 2.0	96.1 ± 2.5

* Recoveries are expressed as a percentage of the label claim (±S.D).
† $n = 6$; for details see text.

General Conclusions

Due to the ease and rapidity with which the standardized analysis scheme can be optimized for a particular problem, the scheme is applied routinely in the authors' laboratory for controlling the label claim of various pharmaceutical dosage forms containing basic drugs (e.g. promethazine in Phenergan® Creme, diphenhydramine in Caladryl® Creme, metoclopramide in Primperan® Solution, ergotamine in Cafergot-PB® Suppositories). The examples described in the present paper typify the authors' experience in applying the strategy to the analysis of basic drugs. The following general conclusions can be made:

(1) Although the octylsulphate extraction technique is sometimes superior to the classical extraction method, it does not allow quantitative yields to be obtained in all instances, even when the internal standard technique is used. Since the HDEHP extraction technique, however, was shown to result in quantitative extraction recoveries in all experiments, even for very hydrophilic drugs, it is the preferred extraction method. Further evidence for this conclusion will be presented in later publications.

(2) The use of an internal standard is generally to be recommended. Its selection is an easy task, however, since it can be based solely on the observed chromatographic properties. Ample experience with the preferred HPLC systems and the elution behaviour of various compounds often allows the choice to be made from substances previously separated, rather than from 'trial and error' experiments.

(3) As could be expected from the criteria on which the selection of the preferred HPLC systems was based, it turns out that an important advantage of the method is the powerful separation ability which characterizes assay methods used in stability-indicating pharmaceutical analyses. Figures 7 and 8 are given as examples. Figure 7 shows the chromatogram of an HDEHP extract of an artificially aged Ben-U-Ron® Suppository, where paracetamol is well resolved from its degradation product *p*-aminophenol. An even more convincing example is illustrated in Fig. 8, which shows the chromatogram of

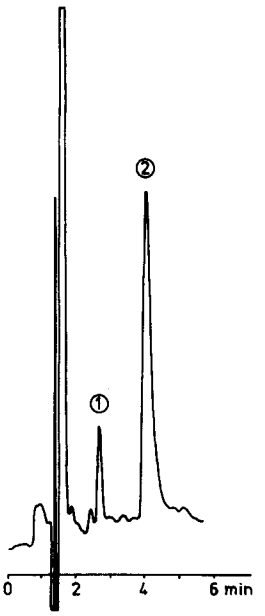

Figure 7
Chromatogram of an HDEHP extract of an artificially aged Ben-U-Ron® Suppository on a Lichrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, *p*-aminophenol (degradation product); 2, paracetamol.

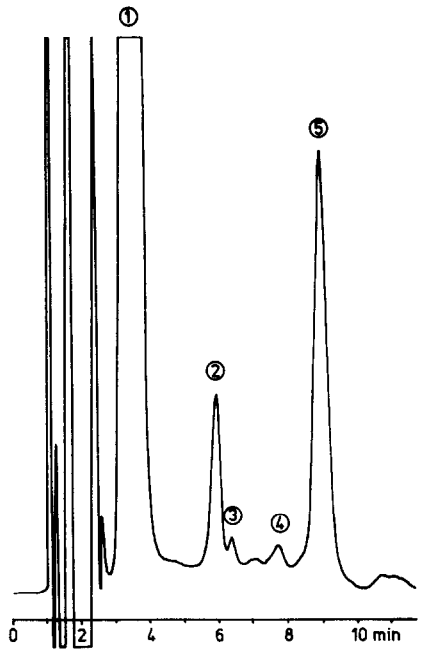

Figure 8
Chromatogram of an HDEHP extract of an artificially aged Cafergot®-PB Suppository on a Lichrosorb-CN column. For chromatographic conditions, see Table 1. Peak identification: 1, caffeine; 2, ergotaminine (degradation product); 3 and 4, degradation products; 5, ergotamine.

an HDEHP extract of a Cafergot-PB® Suppository which had been subjected to accelerated degradation conditions. In particular, the excellent separation of the stereoisomers ergotamine (active product) and ergotaminine (main degradation product) should be noted. Further examples of separations of stereoisomers will be discussed in a later paper.

(4) Selection of the preferred HPLC methods was based on experiments with a MicroPak-CN column [1, 2]. In the present paper, chromatography was performed on a LiChrosorb-CN column. The excellent results obtained with this less expensive column support the authors' belief that their conclusions concerning the preferred HPLC methods are apparently not influenced by brand-to-brand and batch-to-batch differences in materials used for packing the column. It should be noted, however, that important differences were observed in k'-values between a MicroPak-CN column, a LiChrosorb-CN and an Ultrasphere-CN column. The transfer of a mobile phase, optimized for one of these columns, to a different column therefore requires adjustment of the eluent; this can usually be achieved by changing the solvent strength, without affecting the solvent selectivity.

(5) The combination of the HDEHP extraction technique with direct injection of the extract on to a nitrile bonded-phase, combined with a mobile phase chosen from the two preferred eluents, proved to be capable of successful application to all pharmaceutical analyses examined so far. Since column selection is omitted and mobile phase optimization is reduced to fine-tuning one of the preferred eluents, and since the HDEHP extraction technique appears to be generally applicable, much time and work can be saved; this should make the strategy very useful in routine practice.

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