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Analysis of pharmaceutical creams: a useful approach based on solid-phase extraction (SPE) and UV spectrophotometry

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Abstract

Solid-phase extraction (SPE) using C-18, diol and ion-exchange sorbents followed by UV spectrophotometric (conventional and derivative mode) assay was applied to the analysis of basic, acidic and neutral drugs commercially available in creams.

A representative set of drugs (promethazine, chlorhexidine, benzydamine, ketoprofen, ibuprofen, fentiazac, piroxicam, fluorouracil, crotamiton and hydrocortisone acetate) was selected, and for each drug the appropriate SPE conditions (adsorption, washing and elution) were investigated to obtain a practical and reliable sample clean-up. It was shown that the developed SPE procedures were capable of removing interfering cream components (excipients including preservatives) allowing accurate spectrophotometric analyses to be performed. In some applications, derivative spectrophotometry was advantageous over the conventional absorption mode with respect to higher selectivity and versatility.

Keywords: Pharmaceutical creams; Sample clean-up; Solid-phase extraction; Drug analysis; Derivative spectrophotometry

1. Introduction

A number of drugs that possess a variety of pharmacological activities (e.g. anti-inflammatory, antimycotic) are currently used as creams for topical treatment [1,2]. The analysis of these complex formulations can sometimes be performed by simple, non-specific titrimetric methods in non-aqueous solvents [1–3] or in heterogeneous systems [2,4,5], but specific determinations (spectrophotometric and chromatographic methods) are usually required. Spectrophotometric analyses are generally based on chromogenic reactions in order to perform absorbance measurements in the visible region, so avoiding interference from the excipients. Direct UV assays are not always suitable without prior sample clean-up [1,2,5]. Gas chromatography [1,2,5,6], high-performance liquid chromatography (HPLC) [1,2,4,5,7] and thin-layer chromatography (HPTLC) [8–10] are widely applied to the analysis of pharmaceutical creams; the methods usually involve time-consuming and laborious sample clean-up steps, such as liquid–liquid extraction and excipient precipitation by cooling of an appropriate sample solution. When this preliminary clean-up is omitted and the sample solutions are directly subjected to HPLC analysis, intermediate analytical column washings with an organic solvent are recommended [7,11] to remove the liphophilic components of the cream base.

Recently, it has been observed that solid-phase extraction (SPE) can offer the opportunity of simple and practical procedures for

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sample clean-up in the analysis of imidazole antimycotic drugs in creams [12,13]. The role of SPE technique in drug analysis is well known [14–18], but systematic investigations on its application to the analysis of creams have not been reported; some specific applications have been developed only in the HPLC analysis of steroid topical dosage forms using conventional SPE [19–21] and on-line column switching procedures [22–24].

Thus, the aim of this study was to provide convenient and reliable SPE procedures as useful guidelines for sample clean-up in the analysis of pharmaceutical creams. A representative set of basic, acidic, and neutral drugs, belonging to different structural groups (promethazine, chlorhexidine, benzydamine, ketoprofen, ibuprofen, fentiazac, piroxicam, fluorouracil, crotamiton and hydrocortisone acetate) and commercially available as creams were selected. For each drug, the appropriate SPE conditions (sorbent, adsorption, washing, elution) were investigated using UV spectroscopy (conventional and derivative mode) to monitor the development of the SPE method. In this application, UV spectroscopy proved to be a suitable technique to evaluate the performance of the different SPE steps; moreover, with the derivative mode, the residual non-specific matrix absorption was suppressed to allow accurate analyses of commercial creams to be performed.

2. Experimental

2.1. Materials

Promethazine hydrochloride, ketoprofen, piroxicam, chlorhexidine, ibuprofen were obtained from Sigma Chimica (Italy), fluorouracil and hydrocortisone acetate were from Fluka (Switzerland) and benzydamine hydrochloride was from Aldrich (Italy). Crotamiton was kindly supplied by Ciba-Geigy (Italy) and fentiazac was from LPB Istituto Farmaceutico (Italy).

All other chemicals were obtained from Farmitalia C. Erba (Italy). Phosphate buffer solutions (pH 4.5; 7.4; and 8.0) were prepared according to standard methods. Solid-phase extractions were performed on Bond Elut cartridges (500 mg; 2.8 ml) from Analytichem (Varian, USA), using a Baker-10 SPE vacuum manifold connected to a water aspirator.

2.2. Apparatus

All the spectrophotometric analyses were performed on a Jasco Uvidec-610 double-beam spectrophotometer using 1-cm quartz cells with a slit width of 2 nm. Suitable settings were: scan speed 100 nm min^{-1}, chart speed 20 nm cm^{-1}, absorbance scale 0–2.0. For the derivative mode, the $\Delta\lambda$ value and the absorbance scale were selected in accordance with the nature and concentration of the analyte.

2.3. Calibration graphs

Standard solutions of appropriate concentrations in suitable solvents (Table 1) were prepared for each drug. The absorbance at λ_{max} (zero-order spectrum) as well as the selected amplitudes ^1D and ^2D (first- and second-order derivative spectrum, respectively) were plotted against the corresponding concentration to obtain the calibration graphs.

2.4. Solid phase extraction (SPE)

Before use, the SPE columns were properly conditioned as follows: C-18 sorbent by rinsing with 6 ml of methanol; strong anion exchange (SAX) sorbent by rinsing with 6 ml of methanol and then with 3 ml of methanol–buffer solution (pH 8) (1:1, v/v); strong cation exchange (SCX) propylsulphonate (PRS) (propylsulphonic acid) and SCX (benzenesulphonic acid) sorbents by rinsing with 6 ml of methanol and then with 3 ml of buffer solution (pH 4.5); diol sorbent by rinsing with 6 ml of dichloromethane and then with 1 ml of *n*-hexane.

A 3.0-ml aliquot of the sample solution was applied to the appropriate SPE column as described below for each drug.

2.5. Sample preparation

Fluorouracil cream (Efudix)

A sample was dissolved in water–methanol (80:20, v/v) to give a final concentration of about 20 μg ml^{-1} of the drug. A 3.0-ml aliquot was applied to a conditioned C-18 SPE column and the column was then washed with 3.0 ml of water. The filtrate and the washings were combined and subjected to UV analysis by comparison with a standard solution (20 μg ml^{-1}).

Table 1
Data for the calibration graphs ($n = 6$)

Drug	Method	Slope	Intercept	Correlation coefficient	Concentration range (μg ml^{-1})
Fluorouracil	A_{266}	0.0503	−0.0137	0.9997	5–30
	$^2D_{292.8-267.2}$	0.0102	0.0175	0.9980	
Promethazine	$A_{249.2}$	0.0878	−0.0017	0.9994	8.05–20.25
	$^2D_{261.2-251.2}$	0.0211	0.0059	0.9988	
Piroxicam	$A_{325.6}$	0.0649	0.0012	0.9953	10.25–20.5
	$^2D_{371.2-326}$	0.0149	−0.0117	0.9990	
Ketoprofen	A_{266}	0.0658	0.110	0.9995	10.64–35.47
	$^2D_{232-258.8}$	0.0196	−0.0046	0.9999	
Crotamiton	$^1D_{254.4}$	0.0045	−0.0027	0.9998	11.60–46.70
	$^2D_{261.6}$	0.0042	−0.0019	0.9999	
	$^2D_{261.6-247.2}$	0.0082	−0.0072	0.9996	
Benzydamine	$A_{305.6}$	0.0150	−0.0071	0.9976	14.4–43.4
Hydrocortisone acetate	A_{248}	0.0473	−0.0194	0.9956	12–30
	$^2D_{271.6-248.8}$	0.0090	0.0042	0.9971	
Chlorhexidine	$A_{256.8}$	0.0518	−0.0133	0.9995	7.3–36.5
	$^1D_{272-248.4}$	0.0089	0.0005	0.9997	
	$^2D_{259.2-240}$	0.0062	−0.0003	0.9998	
	$^2D_{240-229.6}$	0.0062	−0.0021	0.9996	
Ibuprofen	$A_{271.6}$	0.0151	0.0059	0.9998	6–42
	$A_{263.6}$	0.0197	0.0071	0.9997	
Fentiazac	$A_{300.8}$	0.0186	−0.0094	0.9998	5.1–25.5
	$^1D_{327.6}$	0.0256	0.0038	0.9999	
	$^1D_{254.4}$	0.0344	0.0030	0.9999	

Promethazine cream (Prometazina)

A sample, equivalent to about 1.34 mg of the drug, was dissolved in 100 ml of methanol–0.01 M buffer solution (pH 4.5) (1:4, v/v) and a 3.0-ml aliquot of the sample solution was applied to a C-18 SPE column. The column was washed with two 1-ml portions of the same solvent mixture and the retained drug was eluted with 3 ml of methanol. The eluate was subjected to UV analysis by comparison with a standard solution (15.0 μg ml^{-1}) in methanol.

Piroxicam cream (Feldene)

A sample, equivalent to about 0.143 mg of the drug, was dissolved in 10 ml of dichloromethane–*n*-hexane (2:3, v/v). A 3.0-ml aliquot was applied to a diol SPE column, which was washed with two 1-ml portions of the same solvent mixture and the drug was eluted with 3.0 ml of dichloromethane. UV analysis was performed using a standard solution of 14.0 μg ml^{-1} in dichloromethane.

Ketoprofen cream (Ketofen)

A sample, equivalent to about 0.3 mg of the drug, was dissolved in 20 ml of methanol–0.3 M buffer solution (pH 7.4) (1:9, v/v). A 3.0-ml volume of the solution was applied to a SAX column. The column was washed with two 1-ml portions of the same solvent system and the drug was eluted with three 1-ml portions of methanol–0.01 M buffer solution (pH 4.5) (9:1, v/v). The standard solution contained 14.0 μg ml^{-1} of the drug.

Crotamiton cream (Eurax)

A sample, equivalent to about 4.0 mg of the drug, was dissolved in 100 ml of methanol–buffer solution (pH 4.5) (3:7, v/v) and a 3.0-ml volume of this solution was applied to a C-18 SPE column. The retained drug was then eluted with 3 ml of methanol–buffer solution (pH 4.5) (6:4, v/v) and the eluate was analysed by comparison with a crotamiton standard solution (40 μg ml^{-1}).

Ibuprofen cream (Brufen)

A sample, equivalent to about 3.0 mg of the drug, was dissolved in 100 ml of methanol–phosphate buffer solution (pH 8.0) (1:1, v/v) and a 3.0-ml aliquot was applied to a SAX SPE column. The column was washed with 2 ml of methanol–buffer solution (pH 8.0) (1:1, v/v) and then the drug was eluted with 3 ml of methanol–phosphate buffer solution (pH 4.5) (9:1, v/v). The eluate was analysed by comparison with a standard solution of ibuprofen in the same solvent system (27 μg ml^{-1}).

Chlorhexidine cream (Trust)

A sample equivalent to about 3.0 mg of the drug was dissolved in 100 ml of methanol–phosphate buffer solution (pH 4.5) (1:9, v/v) and a 3.0-ml aliquot was applied to a PRS SPE column. The column was washed with 2 ml of the buffer solution (pH 4.5) and then the drug was eluted with two 1.5-ml portions of buffer solution (pH 7.4). The combined eluates were subjected to spectrophotometric analysis using a standard solution (26 μg ml^{-1}) in the same solvent.

Benzydamine hydrochloride cream (Tantum)

The sample solution was prepared as described for chlorhexidine but a SCX cartridge was used for the SPE.

Hydrocortisone acetate cream (Lenirit)

A sample equivalent to 0.5 mg of steroid was dissolved in 20 ml of *n*-hexane–dichloromethane (7:3, v/v) and 3.0 ml of the resulting solution was applied to a diol SPE column. The column was washed with 2 ml of dichloromethane and the drug was eluted with two 1.5-ml portions of methanol. UV analyses were performed by comparison with a drug standard solution (25 μg ml^{-1}) in methanol.

Fentiazac cream (Norvedan)

A sample equivalent to about 5.0 mg of the drug was dissolved in 100 ml of *n*-hexane–dichloromethane (7:3, v/v) and a 3.0-ml aliquot was applied to a diol SPE column. The column was washed with two 1-ml portions of *n*-hexane–dichloromethane (7:3, v/v) and the retained drug was eluted with two 1.5-ml portions of methanol.

2.6. Assay procedure

The sample solution of each drug was subjected to spectrophotometric analysis (conventional and derivative mode) before and after the SPE step. The absorbances (zero-order spectrum) and the amplitudes ^1D and ^2D (first- and second-order derivative) at the selected wavelengths were compared with the corresponding values obtained from an appropriate standard solution of the drug to calculate the drug content in each sample.

3. Results and discussion

In creams, the active components (basic, acidic and neutral drugs of different polarity) are dispersed in complex emulsions containing hydrophobic and hydrophilic compounds, emulsifying agents and preservatives. Depending on the drug and formulation nature, different SPE processes have been developed in this study. The performance of the SPE methods was first evaluated on standard solutions of each drug and then the suitability of the methods was verified on solutions of commercial samples. To this end, UV spectra (conventional and derivative mode) obtained from the sample solution before and after the SPE step were compared with the spectrum of a standard solution of the drug in the same solvent system.

3.1. Solid-phase extraction (SPE)

The SPE strategy generally comprises the isolation (and concentration) of the analytes from a complex matrix by adsorption onto an appropriate sorbent, the removal of interfering impurities by washing with a suitable solvent system and then the selective recovery of the retained analytes with a modified solvent system of suitable elution strength. If necessary, this process can be modified by selection of sorbent and solvent systems, so that the interfering components are retained by a sorbent and the analytes are then recovered in the filtrate-eluate.

The last approach was found to be effective for the sample clean-up of commercial fluorouracil creams which also contained methyl- and propyl-*p*-hydroxybenzoate. These preservatives exhibit UV spectral properties which can interfere with the spectrophotometric assay of the drug. Thus, using a C-18 sorbent and dissolving the cream sample in aqueous medium (20% v/v methanol), the hydrophobic parabens were

completely retained by the C-18 sorbent while fluorouracil passed through.

When the usual, general SPE process was adopted, the conditions were adjusted to induce the interactions matrix–analyte, analyte–sorbent and analyte–eluent, increasing in that order. Thus, for the analysis of the basic, hydrophobic drug promethazine a C-18 sorbent was used to isolate the analyte from a 20% v/v methanol sample solution; after washing with the same solvent system to remove the excipients, the drug was recovered with methanol. A similar procedure was applied to the clean-up of crotamiton creams. When basic, comparatively hydrophilic drugs such as chlorhexidine and benzydamine were analysed, a SCX packing material was preferred. At pH 4.5 (aqueous medium), the protonated chlorhexidine was retained by a PRS sorbent, while the uncharged excipients passed through the column; subsequent elution with a solvent (pH 7.4) provided quantitative drug recovery. The same procedure was applied to the analysis of benzydamine hydrochloride cream, but an arylsulphonic sorbent (SCX) was used. The last cation exchange material was found to be unsuitable for the extraction of chlorhexidine; the different size and nature of the drugs are probably responsible for different secondary interactions with the aliphatic-aromatic moiety of the SPE sorbent.

Ion-exchange methodology also proved to be suitable for the clean-up of cream samples containing hydrophobic, acidic drugs such as Ketoprofen ($pK_a = 5.9$) and Ibuprofen ($pK_a = 5.2$). The drugs, in the carboxylate form in a basic solvent system, were retained by a SAX sorbent; after appropriate washing to remove the excipients, the drugs were recovered by eluting with an acidic solvent system. This procedure allows elimination of neutral components such as methyl- and propyl-parabens which could interfere with the spectrophotometric determination.

A diol sorbent, previously applied to the sample clean-up of imidazole antimycotic creams [12,13], was found to be suitable for the analysis of formulations containing neutral or acidic drugs of different polarity, such as hydrocortisone acetate, fentiazac and piroxicam. In these applications, the cream sample was dissolved in an appropriate dichloromethane–*n*-hexane mixture, where the dichloromethane–*n*-hexane ratio was suitably adjusted to give weak matrix–analyte interactions and to favour the sorbent–analyte interactions. Under these conditions, the drug was adsorbed onto the diol sorbent while most of the excipients were eliminated; the drug was then recovered by eluting with methanol or dichloromethane.

On the basis of this and previous experience [12,13], the use of a diol sorbent as the first approach for the clean-up of a formulated cream can be suggested. The hydrophobic and acidic–basic properties of the drug as well as the nature of the excipients constitute critical elements for determining the choice of a reversed-phase or ion-exchange methodology for optimisation of the SPE.

3.2. UV spectroscopy

Conventional (zero-order) and derivative (first- and second-order) UV spectroscopy was applied to the analysis of the representative, selected drugs and to monitor the development of the appropriate SPE method. For each drug, linear relationships between the absorption maximum absorbance (zero-order) or the selected amplitudes ^1D and ^2D (first- and second-derivative), and the corresponding drug concentration were found (Table 1). When commercial creams were analysed, the applicability of the different UV methods under different conditions (with and without SPE step) were evaluated. The results are given in Table 2.

Direct conventional spectrophotometric analysis of the sample solutions was not found to be applicable; in each case, inflated assay results were obtained, the extent depending on the excipient structure and the measurement wavelength. This effect was marked for formulations containing parabens (fluorouracil, ketoprofen, ibuprofen, benzydamine) or butylhydroxyanisole (chlorhexidine). Minor effects were observed when the cream base components were of an aliphatic nature; interference with the assay of piroxicam from an aromatic compound (phenylethyl alcohol) was prevented by using high wavelengths for measurements.

Derivative UV spectrophotometric analysis of the sample solution, without a preliminary SPE clean-up did not offer significant improvements in accuracy.

When the commercial cream samples were subjected to the SPE procedures prior to the

Table 2
Assay results [a] for the spectrophotometric (conventional and derivative mode) analysis of pharmaceutical creams before and after SPE clean-up step

Drug (SPE)	Method	Before SPE		After SPE	
		% found	RSD %	% found	RSD %
Crotamiton (C-18)	$^1D_{254.4}$	106.22	2.5	99.41	1.24
	$^2D_{261.6}$	104.13	2.7	98.11	0.73
	$^2D_{261.6-247.2}$	107.86	2.3	98.90	1.71
Ibuprofen (SAX)	$A_{271.6}$	>150.20	– [b]	98.21	0.93
	$A_{263.6}$	>150.15	– [b]	98.29	1.01
Benzydamine (SCX)	$A_{305.6}$	135.70	2.9	98.66	1.20
Chlorhexidine (PRS)	$A_{256.8}$	146.46	2.2	102.95	0.38
	$^1D_{272-248.4}$	140.93	3.0	98.32	0.16
	$^2D_{259.2-240}$	142.31	2.9	98.30	0.29
	$^2D_{240-229.9}$	145.25	2.1	97.83	0.25
Hydrocortisone acetate (2-OH)	A_{248}	110.07	2.3	99.73	0.65
	$^2D_{271.6-248.8}$	90.82	2.6	99.71	0.65
Fluorouracil (C-18)	A_{266}	125.35	3.2	102.62	0.27
	$^2D_{292.8-267.2}$	89.10	3.1	99.80	1.19
Promethazine (C-18)	$A_{249.2}$	140.74	2.3	100.52	1.58
	$^2D_{261.2-251.2}$	107.04	2.8	99.70	2.66
Piroxicam (2-OH)	$A_{325.6}$	103.89	2.5	98.09	0.79
	$^2D_{371.2-326}$	102.96	3.0	98.80	0.70
Ketoprofen (SAX)	A_{260}	127.63	2.4	97.71	0.35
	$^2D_{232-258.8}$	113.76	3.1	98.58	0.88
Fentiazac (2-OH)	$A_{300.4}$	112.81	2.6	98.05	0.89
	$^1D_{327.6}$	86.20	2.9	98.35	0.75
	$^1D_{254.4}$	88.41	2.8	97.50	0.90

[a] The results, expressed as a percentage of the claimed content, are the means of five (after SPE) and three (before SPE) determinations.
[b] (–): not done.

spectrophotometric assay, interference was eliminated and accurate analyses were performed. In general, no significant differences were observed between the conventional and the derivative mode of analysis; slight interference observed in the conventional determination of fluorouracil and chlorhexidine was completely suppressed using the derivative mode. It should be emphasized that crotamiton was analysed only by derivative spectrophotometry. Crotamiton is a typical compound with a UV spectrum characterized by slight shoulders in the usual analytical region 220–300 nm (Fig. 1); using the derivative mode, the shoulders are converted to intense, sharp peaks whose amplitudes can be used for quantitative applications. Moreover it was shown that the SPE method could provide an effective clean-up of the crotamiton sample solution; the derivative spectrum after SPE over the 250–350 nm region was essentially identical to that of an equimolar standard solution. The performance of the adopted SPE procedures can be further illustrated by the examples reported in Fig. 2 (benzydamine hydrochloride), Fig. 3 (hydrocortisone acetate), Fig. 4 (ketoprofen) and Fig. 5 (ibuprofen). Generally, after the SPE step, the UV spectra appear to recover their correct profiles allowing accurate analysis to be performed. It should be pointed out that the method of sample dissolution was chosen to be suitable for the subsequent SPE step; a different method of sample preparation could probably be devised to provide more accurate direct spectrophotometric analyses. The accuracy of the proposed methods was determined by analysing commercial samples spiked with a known quantity of drug; the recoveries were 98.00–100.64%. The precision of the method (SPE and UV assay) was good, as indicated by the RSD (Table 2).

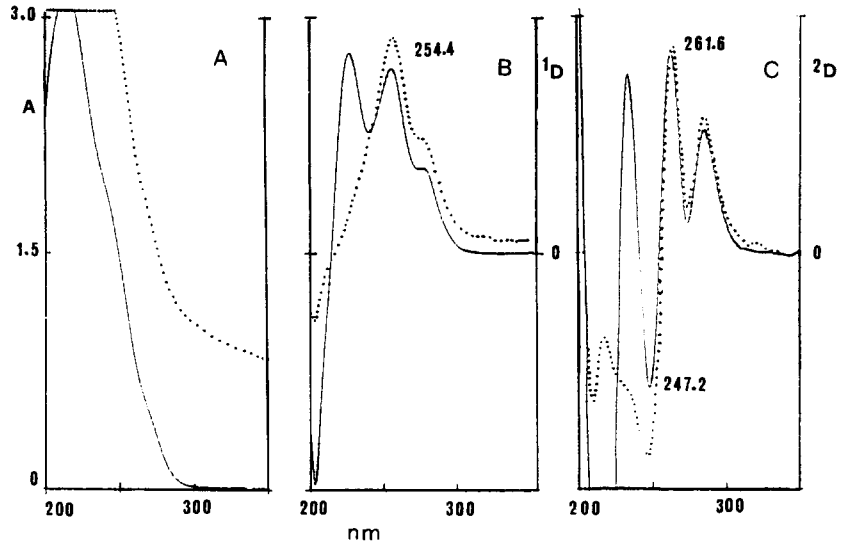

Fig. 1. Zero-order (A), first-order (B) and second-order (C) derivative UV spectra of crotamiton sample solution (45.6 μg ml^{-1}), before (· · ·) and after (——) the SPE (C-18 sorbent) clean-up step.

Because of the generally high recoveries and the good precision (RSD < 1.5%, with the exception of promethazine) which can be attained from experience with the method, SPE-UV spectrophotometry can be considered to be a useful combination for the routine quality control of pharmaceutical creams.

4. Conclusions

Solid-phase extraction (SPE) proved to be an effective tool for performing adequate sample clean-up for the spectrophotometric analysis of drugs in creams. The SPE conditions can be optimized by selecting the appropriate sorbent and eluent systems according to the properties of the drug and excipients (acid–base, lipophilicity). After the SPE steps, both conventional (zero-order) and derivative spectroscopy can be generally applied. The derivative mode, however, offers a more characteristic spectral profile that is useful for drug identification and for improving the accuracy of the method, by using satellite peaks at higher wavelengths and suppressing the residual non-specific matrix absorption. The pro-

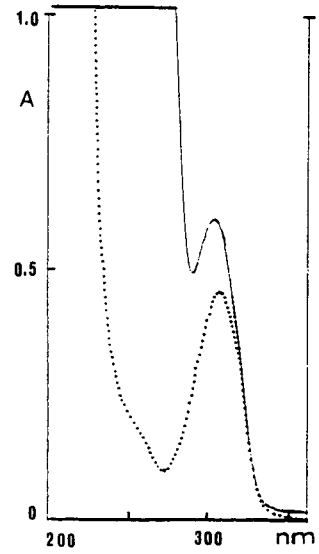

Fig. 2. Zero-order UV spectra of benzydamine sample solution (30 μg ml^{-1}), before (——) and after (· · ·) the SPE (SCX sorbent) clean-up step.

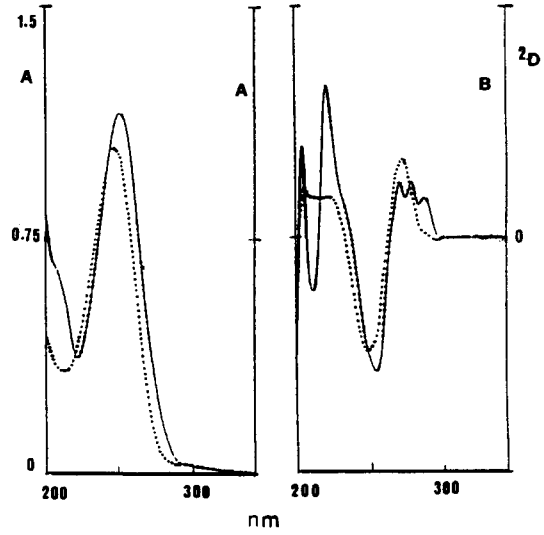

Fig. 3. Zero-order (A) and second-order (B) derivative UV spectra of hydrocortisone acetate sample solution (25 μg ml^{-1}), before (——) and after (· · ·) the SPE (diol sorbent) clean-up step.

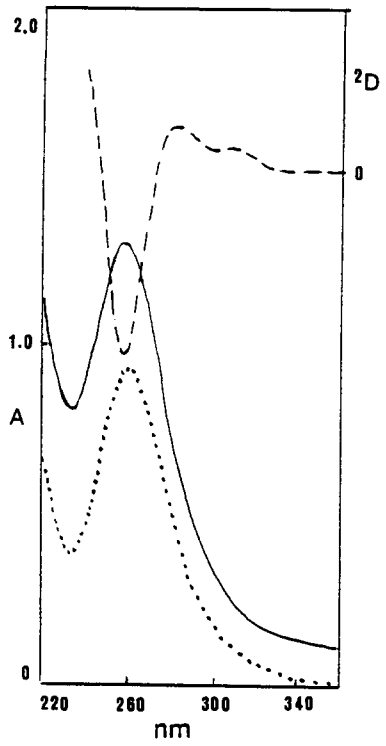

Fig. 4. Zero-order UV spectrum of ketoprofen sample solution (14.2 μg ml^{-1}; pH 7.4) before (——) the SPE (SAX sorbent) clean-up step. Zero-order (· · ·) and second-order (– – –) UV spectra of the solution (pH 4.5) after the SPE step.

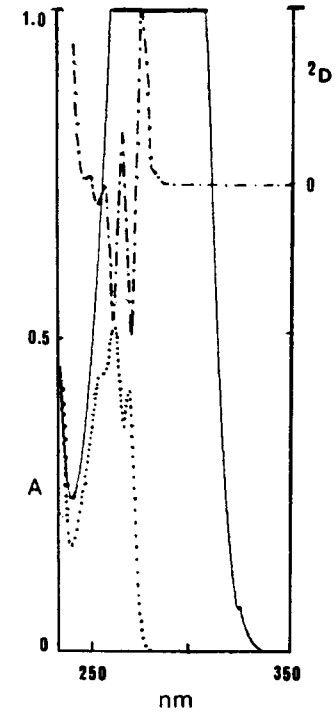

Fig. 5. Zero-order UV spectrum of ibuprofen sample solution (27 μg ml^{-1}; pH 8.0) before (——) the SPE (SAX sorbent) clean-up step. Zero-order (· · ·) and second-order (– · – ·) UV spectra of the solution (pH 4.5) after the SPE step.

posed SPE procedures may also be adopted in chromatographic (HPLC) analyses to avoid overloading and deleterious effects upon the analytical column performance and lifetime.

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References

[1] The United States Pharmacopeia XXII and The National Formulary XVII — United States Pharmacopeial Convention, Rockville, MD, 1990.
[2] The British Pharmacopoeia, Vol. II, HMSO, London, 1993.
[3] F.J. Van der Vaart, A. Hulshoff and A.W.M. Indemans, Pharm. Weekbl. Sci. Ed., 4 (1982) 16–20.
[4] V. Cavrini, A.M. Di Pietra and M.A. Raggi, Int, J. Pharm., 10 (1982) 119–124.
[5] F.J. Van der Vaart, A. Hulshoff and A.W.M. Indemans, J. Pharm. Biomed. Anal., 1 (1983) 507–515.
[6] O.A. Lake, A. Hulshoff and A.W.M. Indemans, Pharm. Weekbl. Sci. Ed., 4 (1982) 43–48.
[7] O.A. Lake, A. Hulshoff, F.J. Van der Vaart and A.W.M. Indemans, Pharm. Weekbl. Sci. Ed., 5 (1983) 15–21.
[8] M. Okamoto and M. Ohta, J. Chromatogr., 369 (1986) 403–407.
[9] L. Zivanovic, D. Zivanov-Stakic and D. Radulovic, Arch. Pharm., 320 (1987) 1183–1185.
[10] J. Sherma, B.P. Whitcomb and K. Brubaker, J. Planar Chromatogr. Mod. TLC, 3 (1990) 189–190.
[11] F.M. Rabel, J. Assoc. Off. Anal. Chem., 64 (1981) 1258.
[12] V. Cavrini, A.M. Di Pietra and R. Gatti, J. Pharm. Biomed. Anal., 7 (1989) 1535–1543.
[13] A.M. Di Pietra, V. Cavrini, V. Andrisano and R. Gatti, J. Pharm. Biomed. Anal., 10 (1992) 873–879.
[14] R.O. McDowall, E. Doyle, G.S. Murkitt and V.S. Picat, J. Pharm. Biomed. Anal., 7 (1989) 1087–1096.
[15] K.B. Furton and J. Rein, Anal. Chim. Acta, 236 (1990) 99–114.
[16] T.R. Krishnan and I. Ibranam, J. Pharm. Biomed. Anal., 12 (1994) 287–297.
[17] M. Moors and D.L. Massart, Trends Anal. Chem., 9(5) (1990) 164–169.
[18] M. Moors, B. Steenssens, F. Tielemans and D.L. Massart, J. Pharm. Biomed. Anal., 12 (1994) 463–481.
[19] M. Zief, L.J. Crane and J. Horwath, Int. Lab., 12 (1982) 102, 104–109.
[20] G. Cortani, Lab. 2000, 2 (1988) 8–9; Chem. Abstr., 113, 120887t.

[21] H. Tokunaga, T. Kimura and J. Kawamura, Iyakuhin Kenkyn, 15 (1984) 87–92; Chem. Abstr., 100, 161853d.

[22] E.J. Benjamin and D.L. Conley, Int. J. Pharm., 13 (1983) 205–217.

[23] D.L. Conley and E.J. Benjamin, J. Chromatogr., 257 (1983) 337–344.

[24] E.W. Smith, J.M. Haigh and I. Kaufer, Int. J. Pharm., 27 (1985) 185–192