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The analytical control of the composition of creams*

F. J. VAN DE VAART[1], A. HULSHOFF†[2] and A. W. M. INDEMANS[2]

[1] *Laboratory of the Dutch Pharmacists, Alexanderstraat 11, 2514 JL The Hague, The Netherlands*
[2] *Pharmaceutical Laboratory, Department of Analytical Pharmacy, University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht, The Netherlands*

Abstract: The analysis without prior sample clean-up of promethazine hydrochloride or clioquinol in commercially available creams has been investigated using UV-spectrophotometry and titrimetric methods. The results were compared with those obtained by GLC and HPLC. Although in some cases the active drug could be determined satisfactorily using the comparatively non-selective UV-absorbance or titrimetric methods, these methods were found to be reliable only when applied to creams for which the qualitative and quantitative composition was completely known. Without prior sample clean-up, these methods will therefore be of interest mainly for process control purposes in manufacturing.

Keywords: *Creams; promethazine hydrochloride; clioquinol; UV-spectrophotometry; titrimetry; gas–liquid chromatography; high-performance liquid chromatography.*

Introduction

From the analytical point of view, creams are among the more complex pharmaceutical dosage forms. They contain lipophilic as well as hydrophilic substances, emulsifying agents and a small amount of preservative. The content of the active component(s) is usually rather low, typically 0.1–3% m/m. Analytical procedures reported for creams often involve gas–liquid chromatography (GLC) or high-performance liquid chromatography (HPLC), taking advantage of the selectivity and sensitivity of these techniques. However, such procedures require highly trained personnel and expensive equipment, so that the cost per analysis may be rather high. On the other hand, UV-spectrophotometric and titrimetric methods are less selective and sensitive but are often less time-consuming and are generally inexpensive. An extra advantage of titrimetry is the fact that it requires relatively few reference standards.

In earlier work the authors have considered the possibility of quantitative analysis of extemporaneously prepared creams using HPLC and GLC as selective techniques [1–3], as well as the less selective methods UV-spectrophotometry [4] and titrimetry [5]. The

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† To whom correspondence should be addressed.

conditions under which the active cream components could be assayed without prior clean-up were carefully investigated. It was found that if: (a) the composition of the cream base is known; and (b) the cream base components are available, so that the possible interference by these substances or their impurities can be investigated; then it can be predicted whether the selectivity of a particular method is sufficient for direct assay of the active component. If not, the kind of clean-up needed to remove the interfering substances can be assessed.

The possibility of applying methods of limited selectivity to the analysis of commercial creams is currently being examined. For these creams, a list of the cream base components can often be found in the compendia (e.g. [6, 7]). The composition therein described can be confirmed by a combination of two HPTLC systems, as recently described [3, 8, 9]. However, the composition of commercial creams, other than the active principles, are not generally known, and this makes the prediction of possible interference less reliable. In this paper, the UV-spectrophotometric and titrimetric determination of promethazine hydrochloride and the UV-spectrophotometric determination of clioquinol in commercial products are described. Some of the results have been compared with those obtained by GLC and HPLC. Extemporaneously prepared creams containing promethazine hydrochloride and clioquinol have also been investigated.

Experimental

All solvents and reagents were of analytical reagent grade. Extemporaneously prepared creams were formulated according to the directions of the Formulary of the Dutch Pharmacists (FNA). The solvent used for the creams was: 96% v/v ethanol–dichloroethane–water (65–25–10, v/v/v).

The composition of the creams was as follows:

Promethazine cream FNA: promethazine hydrochloride 2.25 g, stearic acid 15 g, cetostearyl alcohol 5 g, polysorbate 80.8 g, sorbitan mono-oleate 2 g, sorbic acid 100 mg, 70% v/v sorbitol solution 4 g, triethanolamine 2 g, lavender oil 2 drops, water to 100 g.

Phenergan (Specia): promethazine 2% m/m (as hydrochloride), stearic acid, fatty alcohol, sulphonated fatty alcohol, cholesterol, wool fat, methyl hydroxybenzoate, glycerol, triethanolamine, coumarin, lavender essence [6].

Hydrocortisone and clioquinol cream FNA: hydrocortisone acetate 1 g, clioquinol 3 g, cetomacrogol cream FNA to 100 g.

Betnelan-VC (Glaxo): betamethasone valerate 0.1% m/m, clioquinol 3% m/m, cetomacrogol 1000, cetostearyl alcohol, white soft paraffin, liquid paraffin sodium biphosphate, phosphoric acid, chlorocresol [7].

Celestoform (Warrick): betamethasone valerate 0.1% m/m, clioquinol 1% m/m, soft paraffin, liquid paraffin, cetostearyl alcohol, polyethylene glycol monocetylether, sodium dihydrogenphosphate, phosphoric acid, chlorocresol [6].

Locacorten-Vioform (Ciba-Geigy): flumethasone pivalate 0.02% m/m, clioquinol 3% m/m, cetostearyl alcohol, spermaceti, white soft paraffin, glycerol, sodium laurylsulfate, phenoxyethanol [6].

UV-spectrophotometry

Promethazine HCl. Measurements were carried out using a Perkin–Elmer Model 139 UV-visible double-beam spectrophotometer equipped with a 1 cm flow-cell. Sample

solutions were prepared by dissolving 180 mg of the cream in 100.0 ml of the cream solvent. Standard solutions contained 0.04 mg/ml in cream solvent. Sample solutions and standard solutions were measured against a blank of cream solvent at 305 nm.

Clioquinol. Measurements were carried out using a Uvikon Model 850 double-beam spectrophotometer. An amount of cream containing 3 mg of clioquinol was accurately weighed in a 50 ml flask and dissolved in 5 ml of cream solvent. The solution was made up to volume with glacial acetic acid and filtered if necessary. Standard solutions contained 0.015 mg/ml in the same solvent. The absorbances of the sample solution and the standard solution were measured at 320 nm against a blank of 10% v/v of the cream solvent in glacial acetic acid.

Titrimetry

Titrations with perchloric acid. Potentiograms were recorded with a Metrohm E 536 Potentiograph, using a combined glass–silver/silver chloride electrode system and an automatic 5 ml burette (Metrohm Dosimat E 575). One gram of the cream was dissolved in 35 ml of glacial acetic acid and titrated with 0.05 M perchloric acid. After reaching the equivalence point the titration was stopped and 5 ml of a 3% m/v solution of mercuric acid in glacial acetic acid was added. The titration was continued until the second equivalence point. The content of promethazine hydrochloride was calculated from the difference between these two equivalence points.

Amphimetric titrations. Two hundred milligrams of the cream was dissolved in 5 ml of an acetate buffer solution (pH 2.8), 5 ml water and 30 ml chloroform. After the addition of 5 drops of dimethyl yellow solution (0.5 mg/ml in 90% v/v alcohol), the mixture was titrated with 0.005 M sodium dioctyl sulphosuccinate solution. Near the equivalence point the solution was vigorously shaken after each addition of the titrant.

Gas–liquid chromatography

Promethazine hydrochloride. GLC was carried out using the Intersmat Model 16 DFL-la (Interscience) gas chromatograph equipped with dual flame ionization detectors and fitted with 2 m × 2 mm glass columns, packed with 3% OV-17 on 100–120 mesh Chromosorb WHP. Column temperature was 240°C. Injection and detection temperatures were 300°C and 330°C, respectively. Carrier gas (nitrogen), hydrogen and air flow rates were 20 ml/min, 25 ml/min and 250 ml/min, respectively.

One hundred and fifty milligrams of the cream was dissolved in 5 ml internal standard solution (1 mg/ml chlorpromazine hydrochloride in cream solvent). About 1 μl was injected into the gas chromatograph. In some cases cream samples were subjected to an extraction procedure prior to GLC analysis as previously described [2]. Solutions for calibration contained 0.75 mg/ml promethazine hydrochloride in the internal standard solution.

Clioquinol. A Tracor 560 (Techmation) gas chromatograph was used, equipped with dual flame ionization detectors and fitted with a 1.80 m × 2 mm glass column, packed with 3% OV 101 on 80–100 mesh Chromosorb WHP. Carrier gas (nitrogen), hydrogen and air flow rates were 20 ml/min, 30 ml/min and 300 ml/min, respectively. Column temperature was programmed between 180 and 250°C (at 5°C/min) after an isothermal

period of 3 min. Injection temperature and detection temperature were 230°C and 320°C, respectively.

An amount of cream containing 3 mg clioquinol was dissolved in 1 ml of the internal standard solution (2 mg/ml chlorquinaldol in ethanol). Then 1 ml of water and 1 drop of 2 M sodium hydroxide solution were added. The solution was shaken three times with 5 ml iso-octane. The iso-octane layers were discarded. The aqueous layer was then acidified with 2 drops of 30% v/v acetic acid and shaken with 2 ml of dichloromethane–diethylether (1:4 v/v). The upper organic phase was dried with 2 g of anhydrous sodium sulphate and evaporated to dryness by gentle warming under a stream of nitrogen. The residue was dissolved in 200 μl of toluene–pyridine (9:1 v/v) and 200 μl of acetic anhydride. After heating at 70°C for 1 hr, 1.5 μl was injected into the gas chromatograph. When protected from light these solutions could be kept for several days.

Standard solutions were prepared by evaporating 1 ml of a 3 mg/ml solution of clioquinol in dichloromethane–diethylether (1:4 v/v). The residue was reconstituted in 1 ml of the internal standard solution. This solution was carried through the procedure described above.

High-performance liquid chromatography

A Model 6000 A solvent delivery system, a Model U6K injector, a Microbondapack C_{18} column (30 cm × 3.9 mm i.d.; particle size 10 μm), all from Waters Associates (Milford, MA, USA), and a Pye-Unicam (Cambridge, UK) LC3 variable wavelength detector operated at 254 nm, were used. The mobile phase consisted of methanol–0.5 M phosphate buffer (pH 2.4)–water (60:10:30, w/w/w). The flow rate was 1.0 ml/min. Chromatography was performed at ambient temperature.

Sample solutions contained 2 mg/ml of the cream in the cream solvent. Standard solutions contained 0.05 mg/ml promethazine hyrochloride in cream solvent. Of these solutions aliquots of 10 μl were injected into the HPLC.

Thin-layer chromatography

The compositions of the commercial creams were confirmed by the HPTLC methods recently described [3, 8, 9].

Results and Discussion

Promethazine hydrochloride

The applicability of UV-spectrophotometric and titrimetric determinations of promethazine hydrochloride in creams was tested on a commercial product (Phenergan), and on an extemporaneously prepared cream (Promethazine cream FNA), the results being shown in Table 1. The UV spectrum of promethazine hydrochloride shows a secondary absorption maximum at 305 nm. Neither Promethazine cream FNA nor Phenergan, whose composition [6] was confirmed by TLC, contain substances with significant UV-absorption at this wavelength. Clear solutions of both creams could be prepared using a solvent consisting of ethanol–dichloroethane–water.

Titrimetric methods suitable for the determination of the hydrochlorides of organic bases include: neutralization of the protonated amine-function with e.g. tetrabutylammonium hydroxide (TBAH); titration with perchloric acid in the presence of mercuric acetate, as described in many pharmacopoeias; and amphimetric titration with sodium dioctylsulphosuccinate as the titrant [10]. Promethazine cream FNA and Phenergan both

Table 1
Promethazine hydrochloride content in FNA cream and in Phenergan as determined by various methods

	UV	$HClO_4$	Dioctyl sulphosuccinate	GLC	HPLC
FNA cream	2.33% (2.9%; 5)	2.34% (0.8%; 5)*	2.42% (2.1%; 5)	2.29% (2.3%; 6)†	2.27% (1.9%; 7)
Phenergan	2.29% (1.6%; 4)	—	2.38% (1.9%; 5)	2.25% (3.2%; 6)‡	2.30% (2.7%; 6)
				2.33% (3.8%; 5)†	

* The numbers in brackets are the relative standard deviation and the number of determinations, respectively.
† With extraction clean-up step.
‡ No sample clean-up.

contain stearic acid, which interferes with the titration using TBAH. Triethanolamine is present in both creams and interferes with the perchloric acid titration. If, however, the cream sample solution is titrated first without the addition of mercuric acetate, triethanolamine will be neutralized. After the equivalence point, mercuric acetate is added and the titration continued. The promethazine hydrochloride content can then be calculated from the difference of the two equivalence points. Although this method gave satisfactory results for Promethazine cream FNA, irreproducible results were obtained with Phenergan for reasons not known.

The amphimetric titration of promethazine hydrochloride may suffer from interference by other organic nitrogen compounds. Triethanolamine, however, was found not to influence the results of the titration.

GLC determinations of active substances in creams must often be preceded by a clean-up procedure [2]. However, under the conditions described, promethazine is sufficiently well separated from the cream base components to allow its direct assay in solutions of the creams (Fig. 1). Pre-chromatographic extraction of Phenergan samples yielded the same results, indicating that no interfering peak was present under the promethazine peak in the chromatogram.

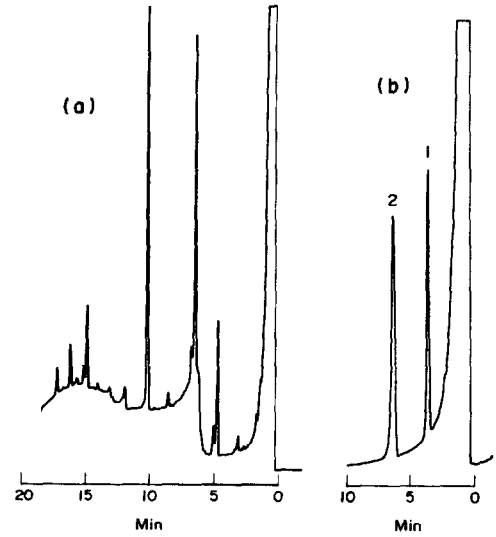

Figure 1
GLC chromatograms obtained by injection of: (a) 30 mg/ml Phenergan in the cream solvent; the oven was temperature programmed from 160 to 330°C at 10°C/min; (b) a Phenergan sample dissolved in internal standard (IS) solution; peak 1 = promethazine, peak 2 = chlorpromazine (IS). For GLC conditions, see text.

The HPLC analysis of promethazine could also be carried out without a clean-up step (Fig. 2). The results given in Table 1 agree with those obtained by the GLC analysis.

For the FNA cream the results obtained with the highly selective chromatographic techniques do not differ significantly from the true stoichiometric promethazine hydrochloride content. It was therefore assumed that this also applies for Phenergan. The results of the UV spectrophotometric determination are not significantly different from those obtained with chromatographic methods. The results of the titrimetric determinations were slightly but significantly higher than the true values (Student's t test; $p < 0.01$).

Clioquinol

The applicability of a UV-spectrophotometric determination of clioquinol in creams, as previously described by the present authors for extemporaneously prepared cream [4],

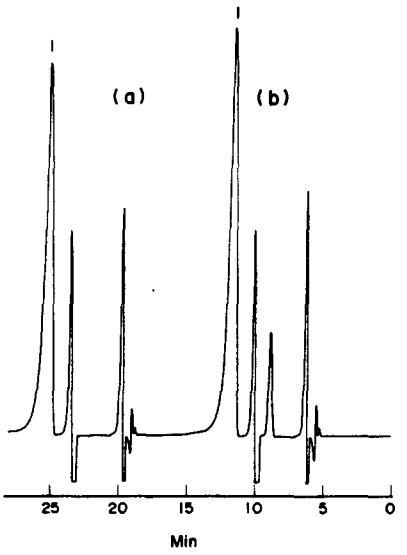

Figure 2
HPLC chromatograms obtained by injection of: (a) a solution of promethazine hydrochloride in the cream solvent; (b) a solution of a Phenergan sample in the cream solvent; peak 1 = promethazine. For details, see text.

was tested on three commercial products. From the composition of the cream bases, as confirmed by TLC, it was concluded that they contained no substances that could be expected to interfere with UV measurements at 320 nm, with the possible exception of aromatic impurities in soft paraffin if present in high amounts. However, commercially available clioquinol is known to contain varying amounts of related 8-hydroxyquinolines, originating from the synthesis: 5-chloro-8-hydroxyquinoline, 5,7-dichloro-8-hydroxyquinoline and 5,7-diiodo-8-hydroxyquinoline. The UV-absorbing properties of these compounds are all rather similar, so that a UV-spectrophotometric determination of clioquinol will only indicate a 'total 8-hydroxyquinoline' content. Nevertheless, if the quality of the clioquinol used to manufacture the cream is known to be high, with only minor amounts of related 8-hydroxyquinolines, or if the same batch of clioquinol can be used to prepare standard solutions, UV-spectrophotometry provides a very convenient method for the quality control of the manufacturing process. This is shown for extemporaneous Hydrocortisone and Clioquinol cream FNA in Table 2.

Table 2
Analytical recovery of clioquinol in FNA cream and in three commercially available creams by UV spectrophotometry and GLC

	UV	GLC
FNA cream	101.8% (0.3%; 6)*	
Betnelan-VC	108.4% (1.8%; 6)	96.3% (2.5%; 4)
Celestoform	102.8% (2.4%; 6)	
Locacorten-Vioform	100.4% (1.5%; 6)	

* The numbers in brackets are the relative standard deviation and the number of determinations, respectively.

Before applying this method to commercial creams, the purity of the clioquinol in these products should be checked. Halogenated 8-hydroxyquinolines can be separated by GLC (Fig. 3). Several methods have been described for the necessary derivatization of these compounds prior to GLC, e.g. silylation [11, 12] and acetylation [13, 14]. In the

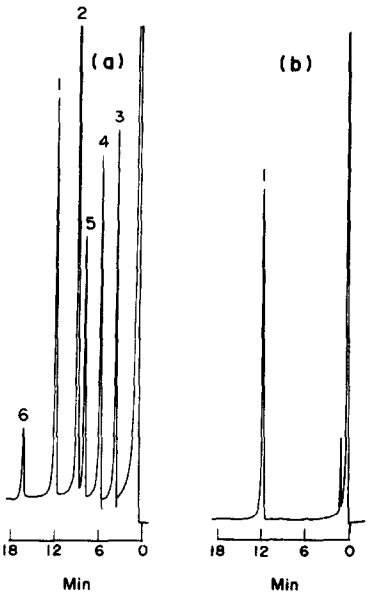

Figure 3
GLC chromatograms obtained by injection of: (a) a mixture of clioquinol (1), chlorquinaldol (internal standard (2), 8-hydroxyquinoline (3), 5-chloro-8-hydroxyquinoline (4), 5,7-dichloro-8-hydroxyquinoline (5) and 5,7-diiodo-8-hydroxyquinoline (6) after acetylation; (b) a Betnelan-VC sample solution after extraction and derivatization as described in the text. For GLC conditions, see text.

authors' hands, acetylation gave the best results. A clean-up procedure appeared to be inevitable, not only because of the occurrence of peaks from cream-base components such as cetostearyl alcohol, but also because of interference by the cream base in the derivatization process itself. It appeared that the creams under study contained only trace amounts of related 8-hydroxyquinolines. The results of the UV-spectrophotometric assay of clioquinol in three commercial creams are given in Table 2. For one cream, Betnelan-VC, significantly higher values were found than the stated clioquinol content, the latter being confirmed by GLC. Upon inspection of the HPTLC chromatograms it was apparent that Betnelan-VC contained much more soft paraffin (containing UV-absorbing compounds) than the other creams.

It can be concluded that analytical techniques with limited selectivity, such as UV-spectrophotometry and titrimetry, are not always suitable for the determination of creams, whose formulation is not exactly known. However, for quality control either during or after the manufacturing process, these simple methods are frequently applicable.

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