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Short Communication

Problems associated with the extraction and analysis of triamcinolone acetonide in dermatological patches*

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Introduction

Over the past 5 years Bristol Myers–SQUIBB DERM has been involved in research and development of novel drug delivery systems with the aim of treating skin disorders such as psoriasis and eczema [1]. An exciting new range of products currently under development in our laboratory is hydrocolloid dressings containing steroids such as triamcinolone acetonide. The patches contain hydrophobic components such as elastomer, adhesive, plasticizer, tackifier and an antioxidant stabilizer in addition to hydrophilic components such as gelatin, pectin and sodium carboxymethylcellulose (NaCMC) which constitute the hydrocolloids.

As part of the development programme for Actiderm plus triamcinolone acetonide (TACA), stability data of the active in the adhesive mass were required for product registration. Therefore, a suitable assay was developed and validated. During initial method development it became apparent that isolation of the active TACA from the hydrophobic and hydrophilic components of the patch and preparation of the sample in a form suitable for chromatographic analysis would prove problematical.

TACA is currently marketed in cream, ointment and lotion formulations and is generally considered to be of intermediate to high potency based on vasoconstriction and clinical studies [2]. Initially, the British Pharmacopoeial method of analysis of TACA in cream and ointment form [3] was employed with the Actiderm plus TACA patch. However, poor recoveries and precision were observed. The USP method of analysis of a commercially available steroid dressing containing flurandrenolide (a steroid analogue of TACA) was also used with Actiderm plus TACA [4]. Again, poor recoveries and precision resulted. One problem experienced when applying the above methods to the present sample was that the hydrophobic moieties of the adhesive mass were not adequately dispersed, thus, the release of TACA from the integral matrix was restricted.

Various techniques of extraction of TACA from the Actiderm adhesive mass were employed, e.g. supercritical fluid extraction (SFE) [5], solid-phase extraction [6] and liquid–liquid extraction. This paper describes the problems associated with method development for the assay of TACA present in the patch formulation and the subsequent development and validation of a specific, robust and reproducible assay.

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Materials and Methods

Reagents and chemicals

The solvents used were: methanol, HPLC grade (Fisons); hexane, HPLC grade (Fisons); chloroform, HPLC grade (Romil); acetone, HPLC grade (Fisons); ethanol, HPLC grade (Fisons); isopropyl alcohol, HPLC grade (Fisons); acetonitrile, HPLC grade (Romil); tetrahydrofuran (THF), HPLC grade (Fisons); de-ionized water; triamcinolone acetonide (Squibb Standard No. 9727; Lot TACA No. 4NN040).

Formulations

These were Actiderm (placebo material) and Actiderm plus TACA (0.1%, w/w).

Solid-phase extraction

Solid-phase extraction was performed on Bond Elut (Analytichem) SiOH, NH_2 and C18 cartridges.

Supercritical fluid extraction/chromatography

Supercritical fluid extraction (SFE) and chromatography (SFC) were performed on a Gilson HPLC system with back pressure regulator, cooling fluid for pumpheads and a column oven. A sample of the Actiderm plus TACA matrix was weighed accurately and placed into a HPLC column. The column was subsequently attached to an SFC instrument and the sample was subjected to extraction with supercritical CO_2 containing 0, 5 and 10% methanol for 5 min each, at elevated temperature (80°C). Eluent was collected by bubbling through methanol (1 ml). Samples were then injected directly into an SFC system. The column used for SFC throughout the investigation was a 10-μm LiChrosorb SiOH 20 × 0.46 cm i.d. column.

Chromatography

HPLC was carried out on a Hewlett Packard model 1090 liquid chromatography system incorporating an HPLC Chem Station linked to an HP Think Jet Printer, using the following chromatographic conditions: stationary phase — 15 × 0.46 cm i.d. Spherisorb 5 μm C8 bonded phase analytical column; mobile phase — methanol–water (70:30); flow rate — 0.5 ml min^{-1}; injection volume — 10 μl; detection — UV, 240 nm.

Analytical procedure

A 2 × 2 cm square from an Actiderm plus TACA patch was weighed into a glass scintillation vial. Hexane (2 ml) was added and the contents were shaken by mechanical shaker for 10 min. When dispersion was complete 8 ml of methanol–water (70:30) were added and mixed thoroughly. The tube was then centrifuged at 2500 rpm for 10 min. Approximately 1 ml of the lower phase was removed and a 10-μl volume was injected directly into the HPLC system.

Results and Discussion

Liquid–liquid extraction

The Actiderm plus TACA patch is composed of hydrophobic components in addition to the hydrophilic hydrocolloids: gelatin, pectin and NaCMC. Clean extraction of a hydrophobic compound such as TACA present at a concentration of 0.1% (w/w) in the Actiderm matrix is dependent on separation of TACA from other hydrophobic components such as elastomers, plasticizers, tackifiers, etc. As these hydrophobic compounds form dispersions or solutions in a number of organic solvents, it was essential to employ a solvent extraction system that extracted the steroid with little contamination from the excipients to give a solution suitable for chromatographic analysis.

Initially, the solubility of TACA in various solvents commonly used in the extraction of drugs from semi-solid or solid formulations was investigated [7]. It was clear from these results that TACA was not readily soluble in aqueous media. However it was reasonably soluble in ethanol, isopropyl alcohol, acetone and chloroform and very soluble in dimethyl formamide.

A number of solvents were examined for the extraction of TACA from the Actiderm patch. Actiderm plus TACA was subjected to sonication with each solvent for up to 2 h. The resulting solutions were either injected directly into the HPLC system with appropriate dilution (methanol, ethanol, isopropyl alcohol, THF, acetonitrile extracts) or evaporated to dryness and reconstituted in methanol–water (70:30) prior to chromatography (acetone extract). The resulting chromatograms clearly demonstrated that dispersion of the adhesive mass was essential for complete recovery of the steroid; THF was the only solvent capable of adequately dispersing the patch matrix. The

Table 1
Recoveries (%) following extraction of TACA from the Actiderm patch with a variety of solvents

Solvent	Recovery (%)
Methanol	<1.5
Ethanol	<1.5
Acetonitrile	<1.5
Isopropyl alcohol	10
Acetone	23
Tetrahydrofuran	100

per cent recoveries obtained with each solvent are shown in Table 1. Although extraction with THF resulted in complete recovery of TACA, the extract contained excipients which resulted in irreproducible results and blockage of the analytical column after 20–30 injections.

Supercritical fluid extraction

SFE has previously been employed for the extraction of compounds that are difficult to extract by conventional techniques [5]. This technique offers a number of advantages over other extraction methods such as reduced sample preparation, speed of operation, ability for direct coupling to a supercritical fluid chromatograph. The steroid dermatological patches allowed the versatility of SFE to be tested. The system used in the extraction is shown in Fig. 1. By employing this technique on the Actiderm plus TACA patch, only 10% recovery of TACA was achieved. Continuing investigations with different modifiers may result in the satisfactory extraction of TACA.

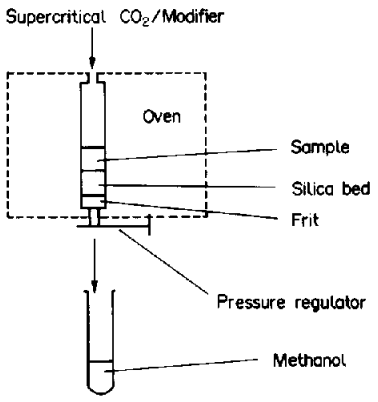

Figure 1
Schematic diagram of supercritical fluid extraction (SFE) of the Actiderm plus TACA dermatological patch. Extraction was performed by elution with (i) CO_2 for 5 min, (ii) CO_2–5% methanol for 5 min, and (iii) CO_2–10% methanol for 5 min.

Solid-phase extraction

Solid-phase extraction employing Bond Elut octadecasilyl amine and silica columns was performed on liquid dispersions of the Actiderm plus TACA patch (THF and hexane dispersions). Although this technique proved to be accurate, the relative standard deviation (RSD) exceeded 2%, rendering the assay unsuitable for use.

The final choice of method

Following attempts to extract TACA from the Actiderm patch by various methods, it was concluded that the most satisfactory results had been obtained by complete dispersion of the patch followed by dilution and chromatographic analysis. The problem, therefore, was in the separation of TACA from hydrophobic excipients, i.e. in the sample clean-up. A number of solvents were investigated for their ability to disperse the patch and provide a "clean" sample for chromatography. All proved unsuccessful with regard to their ability to be applied directly onto the HPLC system. However, hexane completely dispersed the patch matrix and could be subjected to back-extraction. By careful application of the extraction technique, complete recovery of TACA was achieved by back-extraction of a hexane extract with a methanol–water (70:30) solution. When subjected to chromatography this solution was found to be free from excipient contamination and no problems were experienced with column blockage. An added advantage of this method over others was that the polymer backing material of the patch was not destroyed but lay at the interface of the hexane and methanol–water phases and could be easily recovered and weighed. As the backing material is not included in the formulation weight, its weight must be subtracted from the weight of the sample to provide an accurate result for TACA content.

This method exhibited an excellent recovery of 100.4% with a precision (expressed as % RSD) of 1.39%. The assay was linear, the correlation coefficient (r) of peak area response versus % nominal weight of TACA being 0.9991.

Conclusions

Numerous problems were encountered during the development of a stability-indicating assay for triamcinolone acetonide in an integral

adhesive patch formulation. A variety of different techniques were used in order to achieve satisfactory recovery precision, etc. Of the methods used, a liquid–liquid dispersion followed by back extraction proved to be the most successful. One disappointing aspect of the method development was the poor performance of SFE. However, work is continuing in this area to allow complete automation of sample preparation for the Actiderm range of products.

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