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Short Communication

Method validation in pharmaceutical analysis*

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Introduction

One of the most important areas of method development is the validation of the method. Thus, a method must be tested for effectiveness and must be appropriate for the particular analysis to be undertaken. The validation process may vary slightly between laboratories, however, a number of general tests are usually performed; recovery, accuracy, precision, reproducibility, linearity, specificity, limit of detection and quantitation, and ruggedness. Therefore, method development is complete only when the method has been stringently tested and shown to demonstrate acceptable analytical performance [1].

Numerous guidelines are currently available for the validation of analytical methods [2, 3] and with regulatory authorities looking more closely at validation it is vital that procedures become uniform both within a company and throughout the pharmaceutical industry.

The present paper will discuss the Squibb Derm approach to method validation with reference to an HPLC assay of a semi-solid (ointment) formulation containing the steroid tipredane. The tests listed above which are performed during validation will be discussed in detail in addition to other problems experienced during method validation, such as quantitative assay of degradation products in order to demonstrate mass balance during stability studies.

Materials and Methods

Reagents and chemicals

The solvents used were: hexane, HPLC grade (Fisons); acetonitrile, HPLC grade (Fisons); methanol, HPLC grade (Fisons); deionised water. The sample solvent was methanol–deionised water (70:30, v/v).

The tipredane reference standard was SQ27239 (Batch No. NN032NC); degradation product 1, SQ14161 (Batch No. NN016); degradation product 2, SQ14282 (Batch No. NN003); synthesis precursor 1, SQ14150 (Batch No. NN003); synthesis precursor 2, SQ27602 (Batch No. NN002); synthesis precursor 3, SQ27603 (Batch No. NN002).

Chromatography

Instrumentation: Milton Roy Constametric Pump; Gilson 231-401 Autosampler; Spectraflow 757 Detector; Hewlett Packard HP3396A Integrator.

Chromatographic parameters: Stationary phase — Exsil 5 μm C8 15 × 0.46 cm i.d. analytical column (Chromtech); mobile phase — acetonitrile–water (55:45, v/v); flow rate — 1 ml min^{-1}; injection volume — 20 μl; detection — spectrophotmetric at 240 nm.

Results

Degradation of active constituent and specificity of the assay

In order to design a chromatographic system for the analysis of an active component of a pharmaceutical product it is essential to have a good knowledge of; (1) susceptibility of the drug to degradation and its degradation pathway; (2) assay interference by possible degradants or synthesis precursors; and (3) assay interference by chemicals employed in sample

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preparation and excipients present in the formulation.

Degradation products may be formed by acid/base hydrolysis, oxidation, UV irradiation, heat, light, etc. However, it is not within the scope of this paper to discuss in detail the elucidation of degradation pathways. The major degradation products of tipredane (a steroid employed in the treatment of a variety of dermatological disorders) have been characterized as have its synthesis precursors. Therefore, initially, reference standards of tipredane and all known degradants and closely related (in terms of structure) synthesis precursors were chromatographed. Figure 1 clearly demonstrates that tipredane is well separated from any potential interferences. Assay interference by excipients/chemicals used in sample preparation, was investigated by extraction of an ointment placebo. No interfering peaks were observed. Therefore, this method was specific for tipredane.

Recovery

Recovery studies may be performed in a variety of ways depending on the composition and properties of the sample matrix [1, 4]. In the present study, a number of placebo ointments were spiked with an aliquot of tipredane reference standard in hexane and samples were completely dispersed by shaking. The spiked samples were then subjected to the extraction process and recoveries calculated by comparison of peak areas of these samples to the peak areas obtained from a tipredane reference standard solution (Table 1). The results demonstrate that recovery of the analyte from the sample matrix is complete.

Precision

Method precision gives a measure of the method's reproducibility and is usually performed on "real" samples where possible. Tipredane was extracted from the samples of tipredane ointment formulation and the pre-

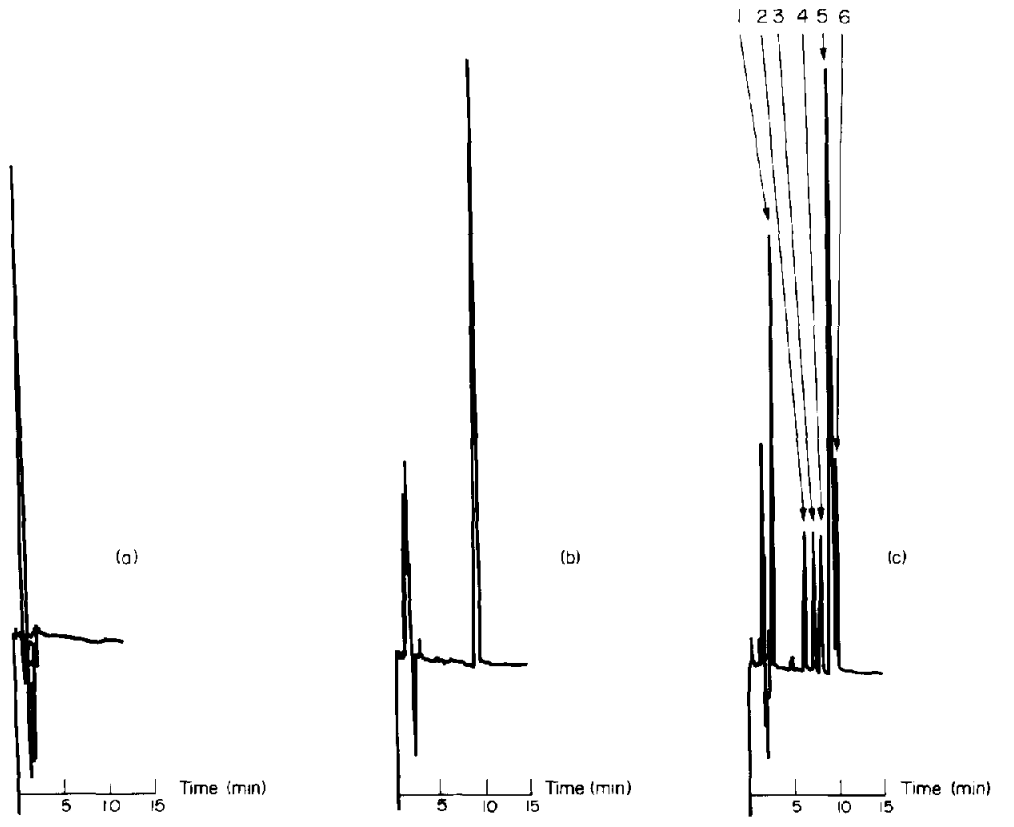

Figure 1
(a) HPLC chromatogram of extracted ointment placebo. (b) HPLC chromatogram of tipredane reference standard. (c) HPLC chromatogram demonstrating separation of tipredane (5) from potential degradation products (1 and 2) and synthesis precursors (3, 4 and 6).

Table 1
Percentage recoveries obtained from nine ointment placebo samples spiked with tipredane at the nominal concentration

Sample no.	Recovery (%)
1	100.2
2	101.4
3	102.7
4	99.7
5	101.4
6	102.5
7	100.9
8	100.6
9	103.3

Mean recovery = 101.4%.

Table 2
Precision of the assay expressed as percentage RSD of 10 "real" samples (response = peak area response divided by weight of sample)

Sample no.	Response
1	10707303
2	10888750
3	10613216
4	10733951
5	10856901
6	10726319
7	10944287
8	10786609
9	10998128
10	10838195

RSD = 1.09%.

cision was calculated as the relative standard deviation (RSD) of the peak areas obtained divided by weight of sample. The results (Table 2) demonstrate excellent precision. Precision experiments give a good indication of the method's performance and should be repeated regularly. Generally, any increase of the RSD above 2% should be investigated and the method completely revalidated.

Linearity

The linearity of the method should be tested in order to demonstrate a proportional relationship of response versus analyte concentration over the working range. It is usual practice to perform linearity experiments over a wide range, i.e. 25–200% of the nominal concentration of analyte [2]. This gives confidence that the response and concentration are proportional and therefore, ensures that calculations can be performed using a single reference standard, rather than the equation of a calibration line.

Linearity experiments were performed on reference standard solutions and spiked placebo samples from 25 to 200% of nominal analyte concentration. Regression analysis of response versus tipredane concentration demonstrated a proportional relationship in both cases (Fig. 2).

Limit of detection and limit of quantitation

Tests to determine the limit of detection (LOD) and limit of quantitation (LOQ) for the procedure are performed on samples containing very low concentrations of analyte or on degradation products. LOD is defined as the lowest amount of analyte which can be detected above baseline noise. LOQ is defined as the lowest amount of analyte which can be reproducibly quantitated above baseline noise.

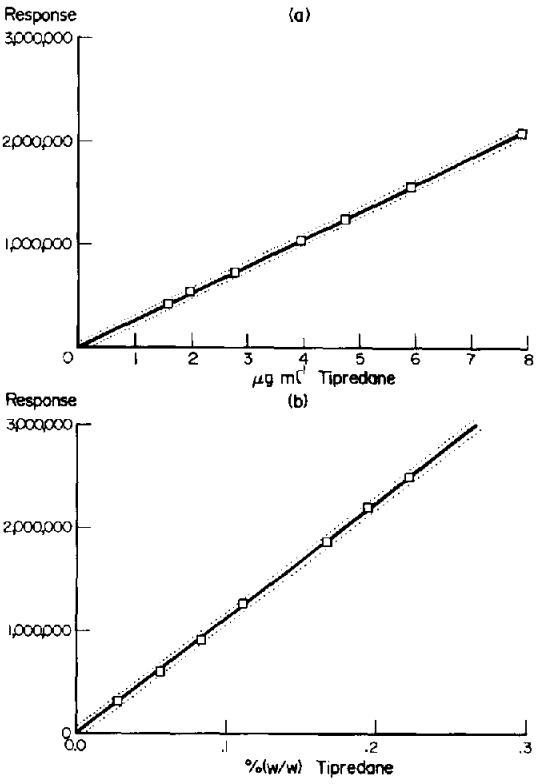

Figure 2
(a) Linearity plot of tipredane standard solution. Peak area response versus tipredane concentration exhibited a proportional relationship with a correlation coefficient (r) = 0.9999. Dotted lines indicate 95% confidence limits. (b) Linearity plot of tipredane in spiked placebo ointment. Peak area response versus amount of tipredane (% w/w) exhibited a proportional relationship with a correlation coefficient (r) = 0.9996. Dotted lines indicate 95% confidence limits.

Table 3
Limits of detection and quantitation of tipredane degradation products (SQ14161 and SQ14282) and synthesis precursors (SQ14150, SQ27602 and SQ27603). Results expressed as percentages with respect to tipredane

Sample no.	LOD (%)	LOQ (%)
SQ14161	0.25	1.5
SQ14282	0.25	1.0
SQ14150	0.25	1.5
SQ27602	0.25	1.5
SQ27603	0.25	1.5

In this study the LOD and LOQ were calculated only for degradation products and synthesis precursors and the results are shown in Table 3. Determination of LOD was based on that amount of sample exhibiting a response three times baseline noise. LOQ was determined by the lowest amount of analyte for which duplicate injections resulted in a RSD of ≤2%. Adequate LOD and LOQ values were obtained for all potentially interfering compounds.

Reproducibility and ruggedness

Reproducibility and ruggedness of the method can be assessed within-laboratory, i.e. by using a number of different analysts within the laboratory that generated the method, and between laboratories, i.e. different laboratories. The reproducibility can be performed by (1) complete validation of the method; (2) assay of the same set of samples by two analysts or laboratories; or by both (1) and (2) and is usually measured by the RSD obtained. In this study, reproducibility was assessed by an inexperienced technician repeating recovery and precision experiments previously performed by the analyst who developed the method. The technician who had had no previous experience of the method was given the standard operating procedure and asked to perform the above experiments. The results (Table 4) clearly demonstrate that the method was reproducible.

Mass balance

In recent years, the regulatory authorities have become increasingly interested in analytical methods, and their validation and have stressed the need for an analytical method or methods to be capable of providing a mass balance throughout a stability study. This means that if a formulation initially contains 0.1% (w/w) of active ingredient, then the value of active plus degradation products (if present) must be maintained at 0.1% (w/w) throughout the period of the stability study. Two or more methods may be required to quantitatively assay the active component and all its degradation products. In this study the HPLC method was capable of resolving all potential degradation products and synthesis precursors in the same run. The assay was also validated for the two main potential degradation products in order that these compounds if present could be quantitatively assayed. This ensured that mass balance was demonstrated throughout the stability study.

Table 4
Reproducibility and ruggedness expressed as RSD of percentage recoveries obtained from eight ointment placebo samples spiked with tipredane at the nominal concentration

Sample no.	Recovery (%)
1	98.8
2	99.1
3	101.2
4	99.5
5	100.6
6	102.1
7	100.3
8	102.0

Mean recovery = 100.5%.
RSD = 1.26%.

Conclusions

The assay of active ingredients in pharmaceutical products and subsequent method validation has become an exacting, complex and time-consuming process in the analytical laboratory. Although general guidelines are followed in terms of parameters tested during method validation, there still remains variation from company to company, and sometimes between different laboratories in a single company. The aim of this paper is to state the method validation process at Squibb Derm. This is not necessarily the "correct" procedure for validation. However, hopefully by presenting methodologies or company philosophy, it will encourage and stimulate more open discussion in this area and may eventually lead to one uniform philosophy throughout the pharmaceutical industry.

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