DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 10(7), 983-990 (1984)

THE DETERMINATION OF MORPHINE IN CAMPHORATED OPIUM TINCTURE BP

C.J. Hinds, D. Johnston* and C.J. Thompson
Sterling-Winthrop Research and Development Division,
Alnwick Research Centre,
Alnwick, Northumberland, England

ABSTRACT

A sensitive liquid chromatographic assay has been developed for the determination of morphine in Camphorated Opium Tincture BP. This assay method is capable of distinguishing morphine from its oxidative and hydrolytic degradation products and does not suffer from interference from a range of related alkaloids. Comparison of this procedure with that given in the BP 1980 has shown that the BP assay gives erroneous results and suffers from a significant positive interference.

INTRODUCTION

During the course of a recent feasibility study Camphorated Opium Tincture BP was included as a component in a cough medicine. As part of this project there was a requirement for a stability indicative assay for morphine in the final product. The BP 1980 assay for morphine[1] in Camphorated Opium Tincture BP is based on a lengthy extraction procedure as developed by Nicholls[2] followed by a colorimetric assay[3]. Attempts to extend this method to the final product proved unsuccessful and it was

* Author to whom correspondence should be addressed.

 0363-9045/84/1007-0983$3.50/0

decided to develop an HPLC procedure which would avoid the necessity for an extensive extraction sequence and provide a rapid sensitive assay method. Two recently published HPLC methods for morphine[4,5] in pharmaceutical products failed to give adequate resolution of morphine from other components in the product before a method, based on a published HPLC assay for morphine and its metabolites,[6] was developed. Application of this method to our product samples gave assay figures for the morphine content which were consistently 110% of those expected from the amount of Camphorated Opium Tincture added based upon the assay of this raw material by the BP 1980 method. When the HPLC assay method was applied to the tincture the results obtained were again consistently 110% of those using the BP 1980 assay procedure. The disagreement between these two methods has been investigated and the work is described.

EXPERIMENTAL

Materials and Reagents - Camphorated Opium Tincture BP and bractium tincture were supplied by William Ransom and Son. Anhydrous Morphine BP was prepared as described in the BP 1980.All other chemicals and solvents were of analytical grade with the exception of the acetonitrile used for chromatography (Rathburn HPLC grade).

Standard Solutions - A stock standard solution of Anhydrous Morphine BP was prepared by dissolving 25 mg of the material in 25 ml of HPLC mobile phase to obtain a concentration of 1 mg/ml. Three, 4 and 5 ml of the stock solution were transferred into three 200 ml volumetric flasks and diluted to volume with the mobile phase to obtain concentrations of 15,20 and 25 ug/ml respectively.

APPARATUS

The modular high performance liquid chromatograph consisted of a constant flow pump (model 6000A Waters Associates); a fixed wavelength UV detector (280 nm, model 440 Waters Associates), a potentiometric recorder, 0.5 cm min^{-1} (Philips PM8241). The column, 0.46 cm (i.d.) x 25 cm was obtained prepacked with Ultrasphere ODS 5 u (Altex) and was maintained at a temperature of 30°C during the analysis.

CHROMATOGRAPHIC CONDITIONS

The mobile phase consisted of a water : acetonitrile mixture (74 : 26 by volume) which was 0.01M in sodium dihydrogen orthophosphate and 0.001M overall in sodium lauryl sulphate adjusted to pH 2.1 with orthophosphoric acid 85%. A flow rate of 1 ml per minute was established.

DEGRADATION EXPERIMENTS ON MORPHINE ANHYDROUS

Degradation of morphine was attempted under the following conditions :-

(1) 25 mg morphine was heated under reflux with 10 ml of 0.1M hydrochloric acid for two hours

(2) 25 mg morphine was heated under reflux with 10 ml of 0.1M sodium hydroxide solution for two hours

(3) 25 mg morphine was heated under reflux with 10 ml of 3%^{w}/v (10 vol.) hydrogen peroxide for thirty minutes

(4) 25 mg morphine was heated under reflux with 10 ml of 5 M sodium hydroxide solution for four hours

(5) 50 mg morphine was heated under reflux with 40 ml of 0.125 M sodium hydroxide solution, whilst passing air through the solution for four hours.

After appropriate dilutions, samples from each of the five treatments were assayed by HPLC.

RESULTS AND DISCUSSION

Separation - Figure 1a shows a typical chromatogram for the quantitation of morphine in the Camphorated Opium Tincture BP. In order to test for interference from related substances a sample of the closely related bractium tincture, which contains a very similar spectrum of naturally occurring compounds but no morphine, was obtained. Figure 1b represents the chromatogram of the camphorated bractium tincture and no interferences in the morphine region are observed. Examinations of the bractium tincture by TLC confirmed the absence of morphine and the similarity of the composition to that
of the Camphorated Opium Tincture.

Standard Curve - Quantitation of morphine in the mobile phase was obtained from a standard curve in which the morphine peak height was plotted against concentration. There is a linear relationship over the range investigated. The standard curve for morphine in the presence of the camphorated bractium tincture was also found to be linear over the same range. The linear regression equations for the two curves were examined using an F

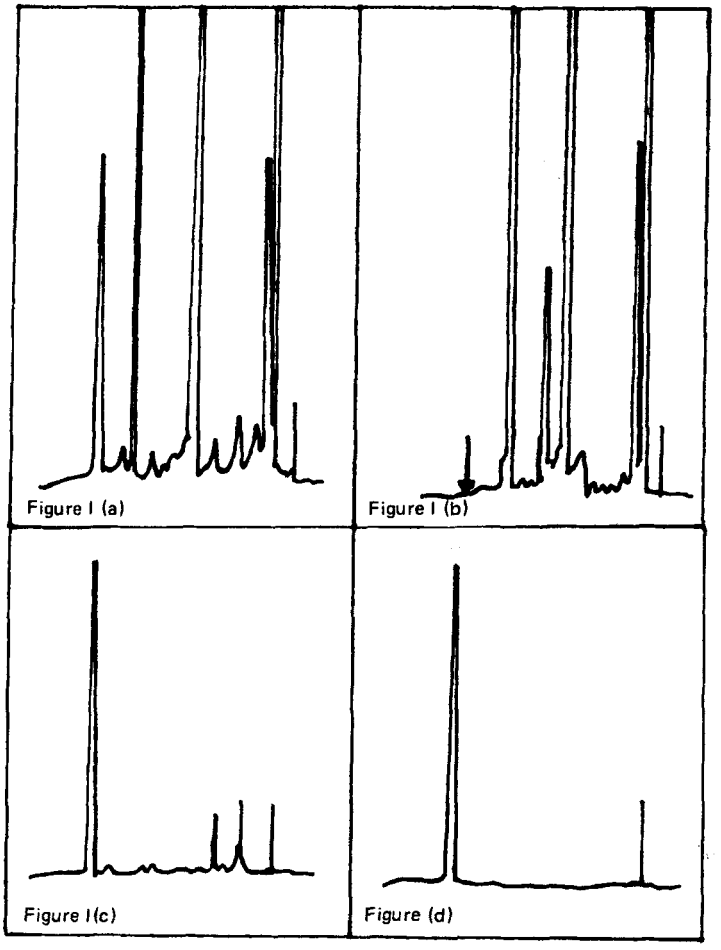

FIGURE 1

1(a) HPLC chromatogram of Camphorated Opium Tincture

1(b) HPLC chromatogram of Camphorated Bractium Tincture

1(c) HPLC chromatogram of Extract from BP assay method

1(d) HPLC chromatogram of Morphine reference

test and no statistically significant differences were observed between the slopes and intercepts of the lines at an α level of $p = 0.05$.

INTERFERENCE FROM OXIDATIVE AND HYDROLYTIC DEGRADATION PRODUCTS

The HPLC chromatograms for the samples of morphine subjected to the degradative conditions are shown in figure 2. As can be seen significant degradation occurred only under relatively severe conditions and in no case was interference in the assay observed.

COMPARISON OF THE HPLC ASSAY RESULTS WITH THE BP 1980 ASSAY RESULTS FOR MORPHINE IN CAMPHORATED OPIUM TINCTURE

When the HPLC results for the batches of Camphorated Opium Tincture were compared with those from the BP colorimetric assay it was observed that the HPLC figures were consistently 110% of the figure obtained by the BP method. In order to investigate this further we examined samples of the Camphorated Opium Tincture which had been passed through the extraction process in the BP procedure by HPLC and by the BP colorimetric assay. We found that while the BP assay result was 100% the assay by HPLC was considerably less and that several additional minor species were present (see figure 1c). Thus it seemed likely that the recoveries obtained using the BP procedure were low but the presence of additional interfering species gave an assay figure which was close to the theoretical value. This supposition was confirmed by applying the HPLC method and the BP 1980 procedure to spiked samples of the bractium tincture when recoveries of 100% were consistently obtained using the HPLC method and recoveries of about 80% were obtained using the BP method. Finally, the two HPLC methods which had been found to be unsuitable for the stability indicative assay of the product were

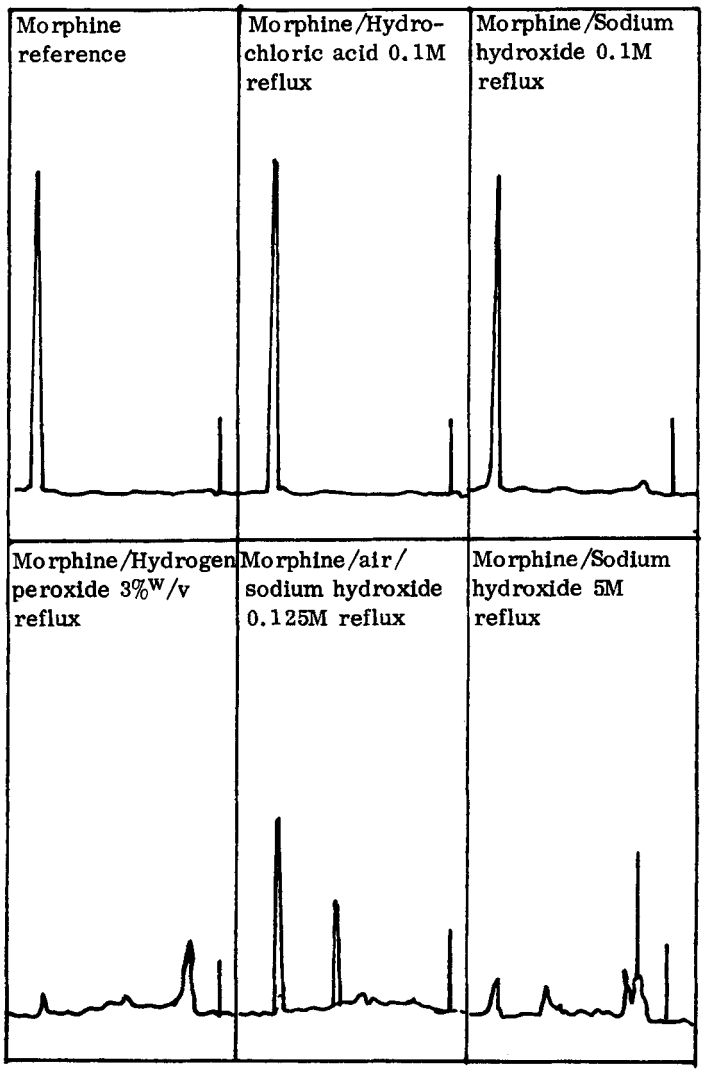

FIGURE 2 - HPLC CHROMATOGRAMS FOR STRESSED SAMPLES OF MORPHINE

applied to the Camphorated Opium Tincture and gave morphine assay figures close to those obtained by our HPLC method i.e. about 10% greater than the theoretical value.

In summary, data has been presented to support the development of a reliable, specific and sensitive high performance liquid chromatographic assay method for the quantitation of morphine in Camphorated Opium Tincture BP.

REFERENCES

1. British Pharmacopoeia 1980

2. J.R. Nicholls Analyst 62 440 (1937)

3. D. Raduleson Bul. Soc. Sti Bucuresti 14 602 (1905)

4. V. Das Gupta J. Pharm Sci 65 1697 (1976)

5. I. Beaumont and T. Deaks J. Chromatog. 238 520 (1982)

6. J-O Svensson et al J. Chromatog. Biomed Applications 230 427 (1982)