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DETERMINATION OF BUFLOMEDIL HYDROCHLORIDE [2',4',6'-TRIMETHOXY-4-(PYRROLIDINYL)BUTYROPHENONE HYDROCHLORIDE] BY REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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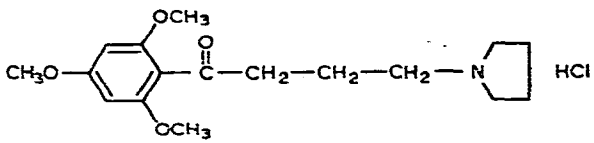
SUMMARY

A simple and rapid method for the determination of buflomedil hydrochloride [2',4',6'-trimethoxy-4-(pyrrolidinyl)butyrophenone hydrochloride] in pharmaceutical formulations by reversed-phase ion-pair high-performance liquid chromatography is described. The sample is dissolved in methanol and 1,3,5-trimethoxybenzene is added as an internal standard. The resulting solution is chromatographed on an octadecylsilane column using ion-pair partition chromatography with lauryl sulphate as the counter ion. A 25-μl injection (containing 3.5 μg of buflomedil hydrochloride) produces a 60% full-scale peak (0.2 a.u.f.s.) at the absorbance maximum (275 nm). The relative standard deviation of the method ranges from 1 to 3%, depending on the particular tablet formulation examined. Excipients present in the preparations do not interfere.

INTRODUCTION

Buflomedil hydrochloride (2',4',6'-trimethoxy-4-(pyrrolidinyl)butyrophenone hydrochloride) is used as a peripheral vasodilator and is presented commercially as 150-mg tablets (Fonzylane®, Lafon and Loftyl®, Abbott Laboratories). An accurate, precise and specific analytical method for the determination of buflomedil hydrochloride in these and experimental formulations is needed.

We have in current use in our laboratories a method for the analysis of



$C_{17}H_{25}NO_4 \cdot HCl$

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buflomedil hydrochloride which involves ultraviolet spectroscopy and requires approximately 1 h to complete. The recent availability, however, of instrumentation for high-performance liquid chromatography (HPLC) has provided a technique of considerable importance in pharmaceutical analysis[1–4]. Recently, a great deal of attention has been devoted to reversed-phase ion-pair partition chromatography. Particularly noteworthy are the publications of Haney and co-workers[5,6], Kissinger[7], Knox and co-workers[8,9] and Waters Associates[10]. We therefore investigated reversed-phase ion-pair HPLC and found it to provide a rapid and accurate method for the analysis of buflomedil hydrochloride. The method, which requires only solution of the sample and dilution to incorporate the internal standard, provides complete separation and quantitation of buflomedil hydrochloride within 10 min. It has been used to assay a number of tablet formulations and to monitor several stability programmes.

EXPERIMENTAL

Chemicals and reagents

Reagents and solvents were obtained from BDH (Poole, Great Britain) (AnalaR grade), except for sodium lauryl sulphate (BDH Biochemicals) and 1,3,5-trimethoxybenzene which was obtained from Aldrich, Gillingham, Great Britain. The buflomedil hydrochloride used was an approved batch of raw material. All chemicals and reagents were used as received.

Standard solutions

The mobile phase was 0.005 *M* sodium lauryl sulphate in methanol–water–glacial acetic acid (70:30:0.1). This was prepared freshly each day and was filtered and deaerated before use.

A stock solution of buflomedil hydrochloride was prepared by dissolving *ca.* 125 mg, accurately weighed, of the drug in methanol and diluting to 250 ml with methanol. A stock solution of 1,3,5-trimethoxybenzene was prepared by dissolving *ca.* 1.34 g in methanol and diluting to 100 ml with methanol.

Apparatus

A Waters Assoc. (Northwich, Great Britain) Model 6000A solvent delivery system or a Metering Pumps (Ealing, Great Britain) Series II Micro Metering pump (fitted with a short stroke mechanism and SS2C pump head, together with a pulse damper and appropriate Bourdon gauges) was used. The pump was set to deliver at 1.0 ml/min and was used in conjunction with a Cecil Instruments (Cambridge, Great Britain) CE 212 ultraviolet monitor operated at 275 nm with a sensitivity of 0.2 a.u.f.s.

Injections were made with a Valco (Field Instruments, Richmond, Great Britain) high-presure injection valve fitted with a 25-μl sample loop. The separation was achieved using a Waters Assoc. μBondapak C_{18} column.

Calibration graph

Aliquots of 20, 30 and 40 ml of the buflomedil hydrochloride stock solution were transferred into separate 100-ml graduated flasks. To each were added 5 ml of 1,3,5-trimethoxybenzene stock solution and the contents diluted to volume with

methanol. The solutions were subjected to chromatography and the heights of the peaks on the resulting chromatograms were measured using a ruler or an integrator. A calibration graph was prepared by plotting the ratio of the height of the peak due to buflomedil hydrochloride to that due to trimethoxybenzene or the ratio of the peak areas *versus* buflomedil hydrochloride concentration. The standard solutions were chromatographed both at the beginning and at the end of a day's run and the results averaged.

Assay procedure

A representative number of tablets were crushed and ground to a fine uniform powder. Duplicate portions, each containing *ca.* 150 mg of buflomedil hydrochloride, were transferred quantitatively into 100-ml graduated flasks and the contents diluted to volume with methanol. The contents of each flask were agitated by immersion in an ultrasonic bath for 5 min. A 10-ml volume of each solution was transferred into a 100-ml graduated flask, 5 ml of trimethoxybenzene stock solution were added to each and the contents diluted to volume with methanol. A 5-ml portion was filtered using a 5-μm porosity Millipore LS filter. These solutions were subjected to chromatography and the ratios of the peak heights or areas on the resulting chromatograms were compared with the calibration graph and the concentration of buflomedil hydrochloride in the sample calculated.

RESULTS AND DISCUSSION

Choice of system

Reversed-phase liquid chromatographic systems are convenient for the analysis of pharmaceutical formulations owing to the ease of sample preparation. Samples are extracted with or dissolved in a suitable solvent such as water, methanol or the mobile phase itself.

Twitchett and Moffat[11], however, have shown that although reversed-phase chromatography using Waters μBondapak columns is satisfactory for neutral and acidic compounds, it is not as good for basic materials. The poor chromatography (tailing peaks, etc.) can be partly overcome by using basic mobile phases such as ammonium hydrogen carbonate in water–methanol mixtures, but the high pH of such phases leads to a short column life.

Haney and co-workers[5,6] showed that acidic substances could be chromatographed efficiently and conveniently using a reversed-phase ion-pairing technique. They indicated that the technique could, by the use of suitable reagents, be extended to the chromatography of basic substances; a prediction amply confirmed by the publications of Knox and co-workers[8,9] and Waters Associates[10]. A consideration of their work led to the preparation of a system consisting of 0.005 *M* sodium lauryl sulphate in a methanol–water mixture acidified with glacial acetic acid. This system has been found in our laboratories to be a convenient and efficient system for the chromatography of bases of different types (quaternary, tertiary, secondary and primary amines) in a variety of formulations, the methanol to water ratio being altered to suit the particular problem[1].

Sodium lauryl sulphate was used as the ion-pairing reagent as it is cheap and readily available in high purity. It has here the further advantage over the other

commonly used reagents, sodium pentane- and heptanesulphonates, that for the analysis to be accomplished in reasonable time a high methanol content in the mobile phase is required. Such a mobile phase gives a markedly lower back-pressure than the 1:1 methanol–water mixtures commonly used with the pentane- and heptanesulphonates. This enables pumping systems of limited capacity to be used with the μBondapak columns, which are prone to give high back-pressures.

Glacial acetic acid was found to be a satisfactory reagent for lowering the pH of the mobile phase to the range (pH 3–5) where organic bases are fully protonated. It may be noted that in other applications where it has been necessary to monitor at much lower wavelengths, *e.g.*, 210 nm, orthophosphoric acid was used instead[1].

The methanol to water ratio was selected such that the analysis was carried out in a convenient time and buflomedil hydrochloride was separated from co-extracted excipients. The system was shown to be suitable for stability studies, *i.e.*, for separating buflomedil from its degradation products, by subjecting buflomedil to severe conditions of stress and examining the reaction products by HPLC. Vigorous refluxing in 1 *M* hydrochloric acid and 1 *M* sodium hydroxide solution for 5–6 h had very little effect on buflomedil when monitored by HPLC, one extra peak being seen on the chromatogram of the acid-treated sample. That these latter results were due to the inherent stability of the drug and not to a lack of resolution of the chromatographic system was substantiated by the similar results obtained on examining the same samples by thin-layer chromatography.

Extraction

Methanol was used as the extracting solvent as buflomedil hydrochloride is readily soluble in it, whereas most of the excipients of the formulations examined are not, *i.e.* water was not used as it also dissolved, for example, various sugars which would be precipitated on the column by the mobile phase. The use of methanol, however, caused the extract to have suspended in it very fine particles of a particular excipient. Although UV-transparent at 275 nm, these particles caused baseline disturbance. This was overcome by filtering the extract prior to chromatography. Experiments showed that the recovery of buflomedil hydrochloride from the various formulations was quantitative.

Sensitivity, linearity and precision

The external standard technique was used initially. The proportionality of the peak height to the amount of buflomedil hydrochloride was measured in the range 0–300 μg/ml. The calibration graph was linear in this range, and the detection limit for buflomedil hydrochloride was 12.5 ng on the column. The reproducibility of the chromatographic procedure was indicated by replicate injections of the same standard solution; the relative standard deviation of the peak height was 0.35% ($n = 10$).

However, the reproducibility was not so good for the formulated samples, and in order to minimize the effects of small procedural variations an internal standard was introduced. 1,3,5-Trimethoxybenzene (retention time, $t_R = 5.0$ min) was chosen as the internal standard as it was eluted before buflomedil hydrochloride (t_R = 9.0 min) and would not, therefore, increase the analysis time; also, 1,3,5-trimethoxy

benzene was found not to be a degradation product of the drug. All formulations were first chromatographed without 1,3,5-trimethoxybenzene to ensure that no interfering peaks were present. A typical chromatogram is presented in Fig. 1.

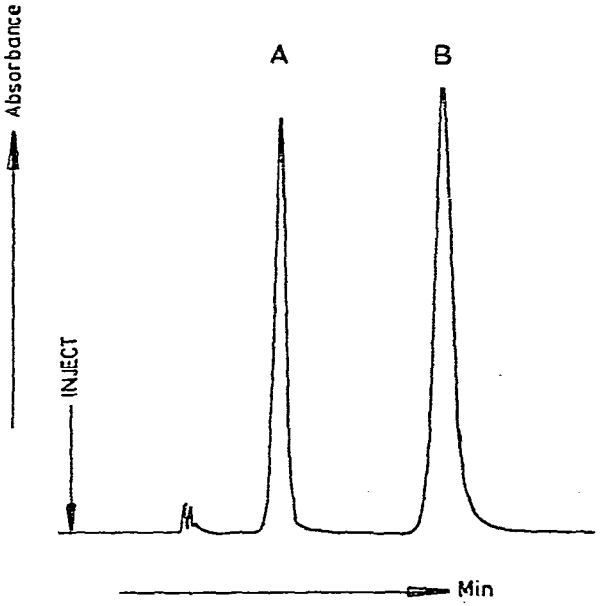

Fig. 1. Typical formulation chromatogram showing (A) 1,3,5-trimethoxybenzene (internal standard) and (B) buflomedil.

A linear response graph of ratio of the peak areas *versus* buflomedil hydrochloride concentration was obtained for the undegraded drug in the range 0–300 μg/ml (Fig. 2). By repeated injection of the same sample solution the relative standard deviation of the chromatographic procedure was determined to be 0.54% ($n = 11$). The relative standard deviation for the whole analysis (weighing, dilution and chromatography) varied from formulation to formulation (see Table I).

To determine the recovery of buflomedil hydrochloride, samples of tablets were prepared containing three different levels of known amounts of buflomedil hydrochloride. The analytical results are presented in Table II. The overall recovery for six samples was 99.6 $\pm$ 1.32%. The small standard deviation demonstrated that buflomedil hydrochloride can be measured with high precision.

In the absence of an integration system, the ratio of peak heights may be used with equally satisfactory results, *e.g.*, relative standard deviation = 0.78% ($n = 10$) for individual tablet assays.

Applications

The method described has been used to monitor the production of batches of various experimental formulations and in the quality assurance of several pilot-scale

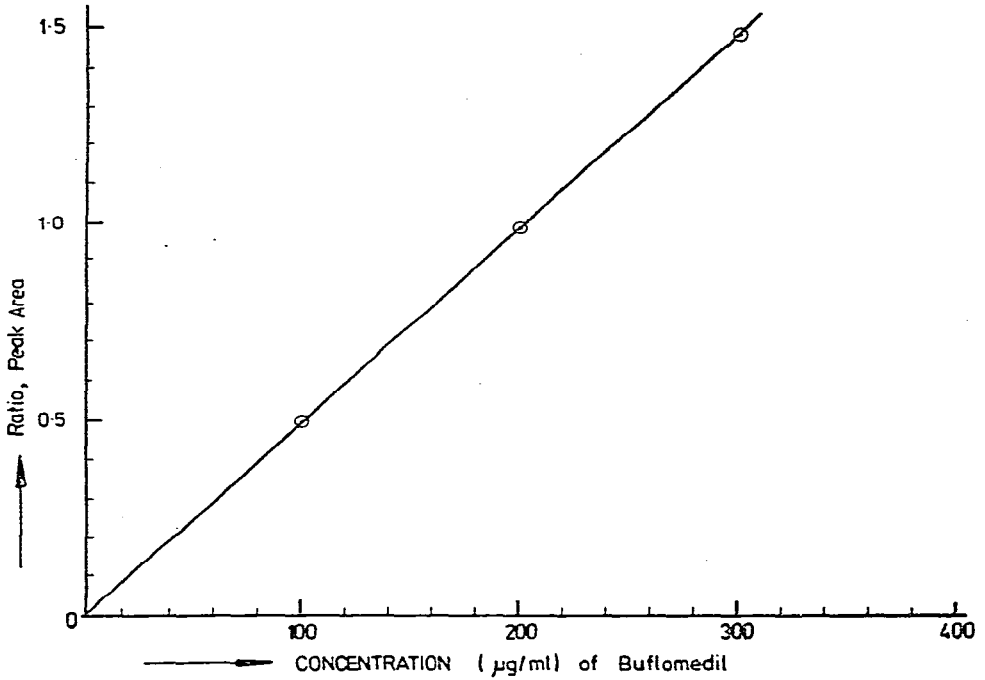

Fig. 2. Response graph of buflomedil hydrochloride concentration *versus* peak-area ratio.

runs (up to 250,000 tablets) of the selected tablet formulations. Where these products have been used in clinical trials it has been used in carrying out individual tablet assays to check on the uniformity of the tablets. The method has also been successfully applied to a number of accelerated stability studies carried out on several

TABLE I

DETERMINATION OF BUFLOMEDIL HYDROCHLORIDE IN TABLET FORMULATIONS

Formulation	*Buflomedil hydrochloride found (mg per tablet)*	*Relative standard deviation (%)*
I	147.0, 148.0, 147.5, 148.0, 148.0, 146.7	± 0.39 ($n = 6$)
II	149, 148, 147, 153, 148, 148, 150.5, 150.5, 148	± 1.27 ($n = 9$)
III	151.4, 152.8, 156.4, 145.9, 145.1, 153.3, 147.2, 146.7, 151.0, 148.5	± 2.49 ($n = 10$)

TABLE II

RECOVERY OF BUFLOMEDIL HYDROCHLORIDE

Amount added (mg)	*Amount found (mg)*	*Recovery (%)*
120	121	100.8
120	120	100.0
150	149	99.3
150	149	99.3
180	181	100.6
180	175	97.2

different tablet formulations. The method is also to be applied, with appropriate modifications, to other pharmaceutical presentations of buflomedil hydrochloride.

Clinical samples (both serum and urine) from patients subjected to buflomedil therapy have also been examined by variations of the method described in this paper. These modifications, together with that used to investigate the serum protein binding of buflomedil, will be described elsewhere[12].

CONCLUSIONS

The reversed-phase ion-pair HPLC method described for the analysis of buflomedil hydrochloride is simple, rapid and accurate. By varying the methanol to water ratio in the mobile phase the retention times can be varied (an increase in the water content resulting in an increase in retention time), and this method should be easily adapted to other buflomedil formulations and to the separation of buflomedil from biological materials. The mobile phase described here has, with minor modifications, been found to be suitable for the analysis of other basic drugs.

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