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DENSITOMETRIC DETERMINATION OF IMPURITIES IN PHARMACEUTICALS PART VI. DETERMINATION OF 4,4–BIS[4–(p–CHLOROPHENYL)–4–HYDROXYPIPERIDINO]BUTYROPHENONE IN HALOPERIDOL

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Abstract: A chromatographic and densitometic method for identification and quantitative determination of 4,4–bis[4–(p–chlorophenyl)–4–hydroxypiperidino]butyrophenone as an impurity in haloperidol pharmaceutical has been developed. The HPTLC plates and chloroform–methanol–ammonium hydroxide 25% (90:9:1) were used for chromatographic separation as stationary and mobile phases respectively. Detection has been carried out in UV at λ=350 nm. The determination could be made directly without preliminary component separation by extraction. Based on the statistical analysis of obtained results, it was found that the new method is accurate and repeatable.

Keywords: determination of impurities, butyrophenones, analysis of pharmaceuticals, 4,4–bis[4–p–chlorophenyl)–4–hydroxypiperidino]butyrophenone, haloperidol

4,4–bis[4–p–chlorophenyl)–4–hydroxypiperidino]butyrophenone (ZH) belongs to butyrophenone derivatives which shows similarity in chemical composition, thus also in physical and chemical properties, to neuroleptic agents used in medical treatment (1). Particular similarity applies to haloperidol (PBF) which is used for treatment of schizophrenia, maniac state and in anaesthesiology for premedication (2).

Analogies in chemical compositions and similarity in physical and chemical properties impede the evaluation of haloperidol purity. This problem has been included into official procedures for haloperidol purity evaluation given in American (3) and European (4) pharmacopoeias, where UV spectrophotometry is used to determine ZH in substance by using differences in absorption spectra for impurities and PBF.

Haloperidol is used in medical treatment in various pharmaceutical forms such as tablets, injections and drops containing also some additives which can impede spectrophotometric determination. Therefore, it is necessary to search for new methods allowing determination of ZH in haloperidol pharmaceuticals.

According to the proven literature, numerous methods have been employed in analysis of 4–fluorobutyrophenon derivatives commencing from thin–layer chromatography (5–9), through gas chromatogrphy (10, 12) to HPLC (13–15) and electrochemical methods (16, 17).

This paper presents a new chromatographic and densitometric method developed for identification of ZH in haloperidol tablets, injections and drops. The separation and detection conditions were chosen to enable us direct determination without using unnecessary extraction procedures having an effect on test duration and result reliability.

EXPERIMENTAL

Apparatus, solutions, reagents

The Kieselgel 60 F_{254} HPTLC – Alufolien plates of aluminium foil coated with silica gel manufactured by Merck were used. Densitometric analysis was carried out on TC Scaner 3 densitometer with Cats 4 software developed by Camag (Switzerland). Linomat IV was used to apply specimens from the same manufacturer.

The solutions were prepared by dissolving appropriate weighed samples in the mixture of chloroform and methanol (1:1 v/v).

Reference solutions:

– 4,4–bis[4–p–chlorophenyl)–4–hydroxypiperidino]butyrophenone (U.S.P.C. Inc., Rockville, MD), solutions from 40 μg/ml to 180 μg/ml.

– Haloperidol standard (USP 23), solution 238 μg/ml.

Test solutions:

Tablets: (Polfa, s. 10989, 20898) containing 1 mg of haloperidol and (Neuraxpham Arzneimittel, s. 942013) containing 4 mg of haloperidol. 5 ml

of chloroform–methanol mixture was added to powdered tablets of weight corresponding to 10 mg of haloperidol. After 15–minute shaking the suspension was centrifuged and supernatant was collected. The solution 50 µl, was applied on each plate.

Injections: (Polfa, s. 30498) containing 5 mg of haloperidol in 1 ml solution. The solution 20 µl, was applied on each plate.

Drops: (manufacturer: ZFA Unia, s. 60989, 20989) containing 2 mg of haloperidol in 1 ml of solution. Testing was carried out directly by applying 50 µl of solution on plates and after extracting 5 ml of solution with three chloroform doses, 5 ml each. The combined chloroform extracts were subjected to vacuum evaporation. The residue was dissolved in 2 ml of chloroform–methanol mixture (1:1 v/v). The solution 20 µl was applied on each plate.

Haloperidol substance (Polfa), a solution containing 5.56 mg of haloperidol in 1 ml of chloroform–methanol mixture (1:1 v/v).

Reagents: Merck reagents of chromatographic purity were used.

Determination conditions

Different volumes of relevant reference and test solutions were applied on plates by linomat to obtain chromatograms in mobile phases at path of 8 cm – 20 cm in length. Chromatograms were dried at room temperature and then subjected to densitometric analysis.

As a result, optimum conditions for chromatographic separation of ZH occurred as impurities in haloperidol and method for its detection were established.

Determination procedure

The reference solutions, 10 µl each, and test solutions in appropriate volumes, as decribed above, were applied by linomat in 15 mm bands on plates of 12×10 cm in size. Chromatograms were developed on the path of 10 cm by using a mobile phase selected experimentally: chloroform–methanol–ammonium hydroxide 25% (90:9:1 v/v). After drying at room temperature, the chromatograms were recorded densitometrically in UV at λ=350 nm.

The spots on chromatograms were indentified by comparing R_f values for reference and tested solutions, whereas its contents was calculated on a computer by comparing appropriate peak areas.

RESULTS AND DISCUSSION

In order to find favourable conditions for chromatographic separation of ZH, the HPTLC plates of high resolution were used as stationary phase that enabled us to separate ZH from PBF by using a mobile phase chosen experimentally: chloroform–methanol–ammonium hydroxide 25% (90:9:1 v/v).

A densitometric analysis of the relationship between absorbance and wavelength (A=f(λ)) revealed a characteristic absorption band with maximum in UV at λ=350 nm for ZH, while in the case of PBF the characteristic absorption band reached its maximum at λ=249 nm. The fact that at λ=350 nm only ZH absorbance is observed allows selective determination of ZH and PBF (Figure 1).

As a result of this analysis the spot locations on the chromatograms were estblished for PBF (R_f ~0.91) and ZH (R_f ~0.76). These results have been confirmed by stain reaction with the Dragendorff reagent (18).

It is important for identification and quantitative determination that, under specified chromatographic separation conditions and densitometric detection conditions, well developed and symmetrical peaks are obtained.

An example of chromatogram and densitogram is presented in Figure 2.

The validation of newly developed method has been carried out by the determination of its detectability, linearity and accuracy.

Based on a series of dilutions, the minimum detectable concentration of ZH was found to be 1.25 µg/ml and its determinability at the level of 2.5 µg/ml.

The linear relationship between the peak area and concentration within the concentration range under investigation (2.5 µg/ml to 40 µg/ml) is presented in Figure 3.

To verify the accuracy of the newly developed method, a model mixture containing 3.20 mg of ZH

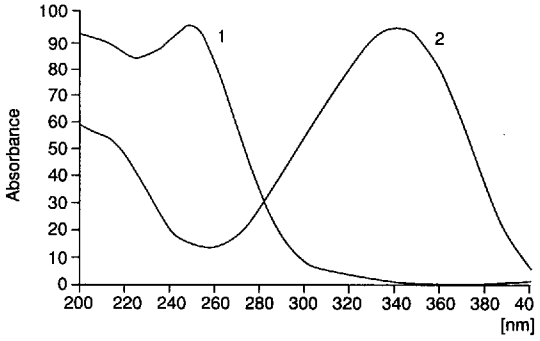

Figure 1. UV spectra obtained densitometrically for appropriate chromatograms: PBF (1) and ZH (2).

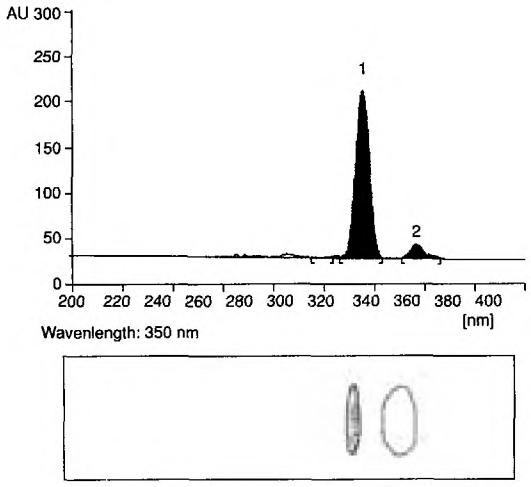

Figure 2. A chromatogram and densitogram recorded at λ=350 nm for ZH (1) and PBF (2). The spot size and location on chromatogram were confirmed by staining with Dragendorff reagent.

in 100 ml of chloroform–methanol mixture (1:1 v/v) was prepared. It was found that, for n=5, the ZH contents x=3.58 mg, S_x=0.27 and $t_{0.95}$= ±0.33. The results we obtained, confirm the high sensitivity and determinability of the new method and guarantee its accuracy and repeatability.

The usability of this method has been verified by determining ZH contents in pharmaceuticals of various forms coming from domestic and foreign manufacturers. The results are presented in Table 1.

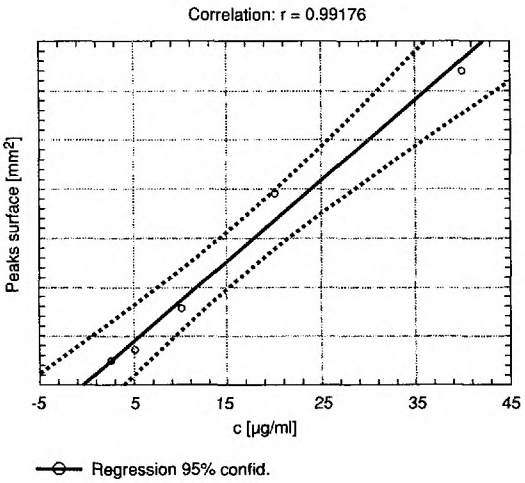

Figure 3. Peak area vs. ZH concentration.

Table 1. Concentration of ZH recalculated to 100 mg of haloperidol, %.

Haloperidol substance	Polish tablets 1 mg/tablet	Imported tablets 4 mg/tablet	Injections 5 mg/ml	Drops 2 mg/ml after extraction	Drops 2 mg/ml without extraction
s.B34233005/97	s.10898	s.942013	s.30498	s.60898	s.60898
$7.48 \cdot 10^{-2}$	$5.65 \cdot 10^{-2}$	$4.23 \cdot 10^{-2}$	$5.35 \cdot 10^{-2}$	$1.57 \cdot 10^{-2}$	$8.69 \cdot 10^{-2}$
$9.20 \cdot 10^{-2}$	$5.69 \cdot 10^{-2}$	$4.33 \cdot 10^{-2}$	$5.50 \cdot 10^{-2}$	$1.85 \cdot 10^{-2}$	$8.64 \cdot 10^{-2}$
$9.70 \cdot 10^{-2}$	$5.57 \cdot 10^{-2}$	$4.34 \cdot 10^{-2}$	$5.13 \cdot 10^{-2}$	$2.00 \cdot 10^{-2}$	$9.71 \cdot 10^{-2}$
$8.20 \cdot 10^{-2}$	$5.17 \cdot 10^{-2}$	$4.40 \cdot 10^{-2}$	$5.33 \cdot 10^{-2}$	$1.72 \cdot 10^{-2}$	$9.60 \cdot 10^{-2}$
$8.00 \cdot 10^{-2}$	$5.58 \cdot 10^{-2}$	$3.84 \cdot 10^{-2}$	$5.44 \cdot 10^{-2}$	$1.92 \cdot 10^{-2}$	$8.50 \cdot 10^{-2}$
$x=8.52 \cdot 10^{-2}$	$x=5.53 \cdot 10^{-2}$	$x=4.14 \cdot 10^{-2}$	$x=5.35 \cdot 10^{-2}$	$x=1.81 \cdot 10^{-2}$	$x=8.31 \cdot 10^{-2}$
$S_x=0.9 \cdot 10^{-2}$	$S_x=0.21 \cdot 10^{-2}$	$S_x=0.22 \cdot 10^{-2}$	$S_x=0.14 \cdot 10^{-2}$	$S_x=0.17 \cdot 10^{-2}$	$S_x=0.58 \cdot 10^{-2}$
$t=\pm 1.13 \cdot 10^{-2}$	$t=\pm 0.26 \cdot 10^{-2}$	$t=\pm 0.27 \cdot 10^{-2}$	$t=\pm 0.18 \cdot 10^{-2}$	$t=\pm 0.21 \cdot 10^{-2}$	$t=\pm 0.72 \cdot 10^{-2}$
	s.20898			s.20898	s.20898
	$5.10 \cdot 10^{-2}$			$1.81 \cdot 10^{-2}$	$7.67 \cdot 10^{-2}$
	$5.16 \cdot 10^{-2}$			$1.96 \cdot 10^{-2}$	$7.73 \cdot 10^{-2}$
	$4.62 \cdot 10^{-2}$			$2.01 \cdot 10^{-2}$	$7.53 \cdot 10^{-2}$
	$5.40 \cdot 10^{-2}$			$1.71 \cdot 10^{-2}$	$8.12 \cdot 10^{-2}$
	$5.10 \cdot 10^{-2}$			$2.14 \cdot 10^{-2}$	$8.28 \cdot 10^{-2}$
	$x=5.08 \cdot 10^{-2}$			$x=1.93 \cdot 10^{-2}$	$x=7.87 \cdot 10^{-2}$
	$S_x=0.28 \cdot 10^{-2}$			$S_x=0.17 \cdot 10^{-2}$	$S_x=0.32 \cdot 10^{-2}$

x = mean value, S_x = mean standard deviation, $t_{0.95}$ – confidence level

CONCLUSIONS

It was found that all pharmaceuticals under examination were contaminated with ZH. The content of this impurity in injections and tablets manufactured in Poland was similar to those of imported tablets (Table 1). Haloperidol substance and drops contained ZH in higher concentrations as compared to other drug forms under examination. However, these differences are not significant as they are within the permissible limits (3, 4). The observed differences shall be assigned to quality of original substance used for preparation of given drug form.

The significant differences in ZH contents were found in drops directly analysed and after preliminary ZH separation by extraction. The results obtained with chloroform extraction are quite different to those of direct determination. This casts doubts on the reliability and accuracy of the results obtained by extraction methods for preliminary ZH separation. The results presented in Table 3 provide an evidence for direct determination of ZH.

The tests we carried out clearly indicate that the newly developed chromatographic and densitometric method can be useful for identification and determination of ZH impurities in various drug forms. No preliminary extraction is required under specified conditions, since chromatographic separation and proposed detection method eliminate an effect of the concomitant substances on the results of determination.

The results of statistical analysis presented in Table 1 and Figures 1 to 3 have revealed the high sensitivity, good precision, repeatability and accuracy of the new method.

Finally, it should be noted that the newly developed method for determination of ZH can be used in routine analysis for drug purity assessment due to its simplicity and high accuracy that guarantees reliable results.

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