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# A note on the colorimetric determination of butyrophenones with 3,5-dinitrobenzoic acid

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Butyrophenones react with 3,5-dinitrobenzoic acid in an alkaline medium with the formation of a red coloured complex. This reaction can be used for the quantitative determination of these drugs in pharmaceutical preparations. The influence of the ethanol concentration, the alkalinity and the reagent concentration was investigated separately. The results show that the proposed method is suitable for the assay of solutions and tablets. It requires less time than existing methods.

Few publications have described methods for the quantitative determination of butyrophenones in pharmaceutical preparations. In 1961, Demoen published an ultraviolet assay method for haloperidol which, though sensitive, is not specific. He also described a titration in acetic acid; but a large amount of drug is required; and a colorimetric method for the determination of haloperidol in urine based on the reaction of haloperidol with methyl orange. Demoen's ultraviolet method has also been used by Janicki, Brenner & Schwartz (1968) to determine droperidol in combination with fentanyl. In our laboratory, we (Van den Bossche, Haemers & De Moerloose, 1969) developed a method for the quantitative determination of fluorinated drugs in pharmaceutical preparations based on the colorimetric determination of the fluoride ion, formed after combustion in a Schöniger flask, with alizarine fluorine-blue. This method has also been used to assay butyrophenones. Soep (1961) determined butyrophenones in biological fluids by fluorine determination; he found it impossible to determine butryrophenones with the usual ketone reagents and we can confirm this. Also, the colour reaction of haloperidol with 1,3-dinitrobenzene, described by Thomas & Dryon (1967) cannot be used for the direct determination of butyrophenones in aqueous solution.

Tattje (1958) described an assay method for $\alpha$-$\beta$ unsaturated ketones (thujone, pulegone, piperitone) in volatile oils with 3,5-dinitrobenzoic acid in alkaline medium. As all butyrophenones react in the same way, we sought the most favourable reaction conditions using a solution of the butyrophenone pipamperone as hydrochloride.

## EXPERIMENTAL AND RESULTS

In the examination of the reaction conditions, the influence of ethanol concentration (20–70%), strength of sodium hydroxide (0·2–0·9N) and concentration of 3,5-dinitrobenzoic acid (0·5–3·0%) was assessed. Whatever the concentration of 3,5-dinitrobenzoic acid and the alkalinity, maximum extinction occurred with an ethanol concentration of 30% v/v. Although the sensitivity of the reaction is optimal at that concentration, the colour is stable for too short a time and because of this an ethanol concentration of 40% was chosen since the colour stability is much increased while the sensitivity diminishes only slightly. Increasing the alkalinity increases sensitivity and reaction velocity, the colour stability however diminishes so a final concentration of 0·6 N was chosen. Colour stability is also affected by the reagent and as concentration increases stability diminishes. A final concentration of 1,5% 3,5-dinitrobenzoic acid was found to be most suitable.

Other butyrophenones, required the same optimal reaction conditions.

The absorption spectrum of the red coloured complex shows a maximum at 525 nm. All the butyrophenones examined had maximum absorption at the same wavelength. Furthermore, all butyrophenone-3,5-dinitrobenzoate complexes obey Beer's law in a concentration range from 0 to 1 mg.

*Method*

*Reagents.* Ethanol 96% (aldehyde-free: U.S.P. XVII); 3,5-dinitrobenzoic acid (7·5% in ethanol 96%); 3 N sodium hydroxide in water.

*Aqueous solutions.* Dilute the sample of butyrophenone with water to give a 0·05 to 0·25 mg/ml solution. Pipette 4·0 ml into a test-tube and add consecutively aldehyde-free ethanol (2·0 ml) colour reagent (2·0 ml) and 3N sodium hydroxide solution (2·0 ml). Mix, and measure the extinction after 8 to 12 min in 1 cm cells at 525 nm. Calculate the concentration of butyrophenone by comparison with the extinction of a similarly-treated standard solution or by means of a calibration curve. In this way, solutions for oral or parenteral administration and tablets containing water-soluble butyrophenones, such as pipamperone HCl, can be determined.

*Ethanolic solutions.* Dilute the sample of the butyrophenone with the aldehyde-free ethanol to obtain a 0·1 to 0·5 mg/ml solution. Pipette 2·0 ml into a test-tube and add consecutively water (4·0 ml) colour reagent (2·0 ml) and 3N sodium hydroxide solution (2·0 ml). Measure extinction as under aqueous solution above. In this way, tablets containing ethanol-soluble butyrophenones, such as haloperidol, can be assayed.

Using the proposed method, we have examined pharmaceutical preparations containing butyrophenones. Table 1 contains the results.

Table 1. *Results obtained by the proposed method*

| Sample | Found (%) |
|---|---|
| Azaperone injection 40 mg/ml | 98·1 |
| Benperidol drops 1 mg/ml | 99·3 |
| Droperidol injection 2·5 mg/ml | 98·6 |
| Fluoanisone drops 25 mg/ml | 100·3 |
| Haloperidol drops 2 mg/ml | 99·8 |
| Haloperidol tablets 0·5 mg | 100·7 |
| Pipamperone 2 HCl tablets 40 mg | 98·5 |
| Trifluperidol HCl drops 1 mg/ml | 99·3 |

The results prove that the proposed method is suitable for the determination of butyrophenones in pharmaceutical preparations. It can be used for aqueous as well as for alcoholic solutions. Preliminary extraction of preservatives, such as methyl- or propylparaben, is unnecessary. The method is accurate (standard deviation: 0·9%) and can be executed in minimum time.

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