Spectrophotometric determination of some drugs containing a tertiary amine group

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Base-catalysed condensation of mixed anhydrides of organic acids where a tertiary amine functions as the basic catalyst, yields a highly coloured condensation product and is specific to tertiary amines or their chloride salts (Roeder, 1941; Feigl, 1966). This reaction is suitable for the spectrofluorometric determination of tertiary amines (Thomas, 1975). The present communication reports on this reaction as a spectrophotometric method for the analysis of promethazine, pilocarpine, emetine and noscapine hydrochlorides and ampicillin trihydrate. Of these, pilocarpine hydrochloride has been determined previously by spectrofluorimetry using the same reaction (Thomas, 1975).

Malonic acid in acetic anhydride (MAA reagent 10%) has been previously described.

For all amines except ampicillin trihydrate the colour is developed by heating the drug substance (2–3 mg) with the MAA reagent (3 ml) for 15 min at 80°. The solution is then made up to 25 ml with ethanol. A suitable aliquot (normally 0·1–0·2 ml) is then diluted to 10 or 25 ml with ethanol and the absorbance measured at 333 nm against an ethanol blank. For ampicillin trihydrate, 3 mg are reacted with 1 ml only of the MAA reagent at 100° for 30 min. The reaction mixture is then treated as above. The pKa value of ampicillin trihydrate (pK = 2·52) is lower than any of the other amines studied (pK range, 6·4–9·1) (Perrin, 1965) thus suggesting that the rate of the condensation reaction is dependent on the basicity of the tertiary amine catalyst.

For colour development the amine also must be soluble in the MAA reagent, but of nine so far investigated, only thioguanine was insoluble and thus did not catalyse the condensation reaction.

Noscapine hydrochloride B.P. (Macfarlan Smith, Edinburgh) pilocarpine hydrochloride (BDH Chemicals Ltd), emetine hydrochloride (Koch-Light Laboratories), promethazine hydrochloride (gift from May and Baker Ltd.) and ampicillin trihydrate (gift from Beecham Research Laboratories) were purified by recrystallization. Other reagents were as previously described.

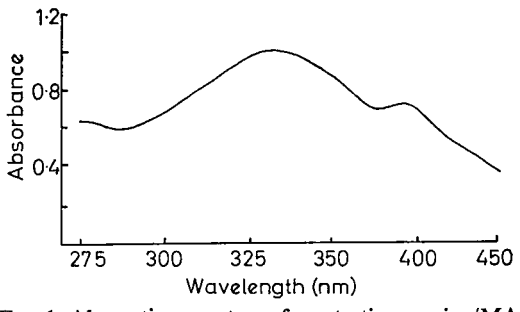

FIG. 1. Absorption spectrum for a tertiary amine/MAA complex, where the tertiary amine is emetine hydrochloride.

Table 1. *Spectrophotometric determination of some tertiary amines.*

	Limit of detection (μg ml^{-1})	Molar absorptivity (litres mole^{-1} cm^{-1})	Standard deviation[a] (μg ml^{-1})
Pilocarpine hydrochloride	0·02	$1{\cdot}90 \times 10^5$	0·04 (0·93)
Emetine hydrochloride	0·03	$1{\cdot}94 \times 10^5$	0·05 (0·6)
Promethazine hydrochloride	0·03	$1{\cdot}2 \times 10^5$	0·01 (1·4)
Ampicillin trihydrate	0·28	$2{\cdot}4 \times 10^4$	
Noscapine hydrochloride	0·01	$1{\cdot}5 \times 10^5$	0·01 (0·34)

a. Ten (emetine hydrochloride) and 5 (noscapine hydrochloride) separate solutions, with average concentrations indicated in parenthesis, analysed. Fifteen replicate solutions analysed for pilocarpine and promethazine hydrochlorides.

The absorption spectrum for all five amine/MAA complexes was identical and is shown in Fig. 1. The limit of detection (Wilson, 1961) for the amines is given in Table 1. Dilute solutions ($\simeq 1{\cdot}0$ μg ml^{-1} of the complex) are stable for 90 min but show a gradual decrease in absorbance over this period ($\leqslant 5\%$).

The results of analysis of several pharmaceutical preparations are shown in Table 2.

When the amine to be determined was present as a solution in sterile water (promethazine hydrochloride)

Table 2. *Analysis of pharmaceutical formulations.*

Alkaloid	% Stated (w/v)	% Found (w/v)	RSD[a](%)
Pilocarpine hydrochloride eye drops	2·0	2·1	2 (5)
Coscopin linctus (noscapine)	3·26	3·23	4·2 (3)
Promethazine hydrochoride injection	2·5	2·46	6·5 (10)

a. RSD = Relative standard deviation.

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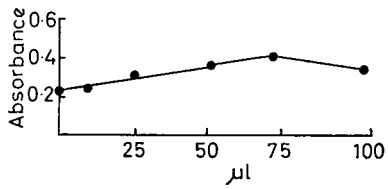

FIG. 2. Effect of adding water (1 µl) on the absorbance of pilocarpine hydrochloride/MAA complex.

or 0·002% benzalkonium chloride (pilocarpine hydrochloride), a suitable volume equivalent to 2–3 mg of the amine was pretreated by adding 1 ml of acetic anhydride before heating with the MAA reagent. Water affects the absorbance of the condensation product, but in a reproducible manner (Fig. 2) and provided the amount of water in the calibration standard and assay sample is the same, accurate results may be obtained (Table 2).

Typically, 50 µl aliquots of pilocarpine hydrochloride eye drops were taken and 50 µl of 0·002% benzalkonium chloride solution was added to the pilocarpine hydrochloride standard before reaction. Alternatively the solvent can be evaporated and the MAA reagent added to the dry residue. If the amine is present in a complex mixture such as a linctus, or is in tablet form, preliminary extraction of the amine is necessary. Noscapine hydrochloride was extracted by the method of the British Pharmaceutical Codex (1968).

Table 3. *Comparison of limits of detection for MAA/-spectrophotometric method with some other spectrophotometric methods.*

	Literature value (µg ml^{-1})	This study (µg ml^{-1})
Ampicillin trihydrate	2[a]	0·28
Pilocarpine hydrochloride	0·1[b]	0·02
Promethazine hydrochloride	60[c]	0·03

a. Celletti, Moretti & Petrangeli (1972).
b. Repta & Higuchi (1971).
c. Tarasiewicz (1972).

The method presented here compares well with other spectrophotometric methods of analysis for the various amines in terms of the speed of the reaction and the limits of detection (Table 3). A lowering of the limit of detection by at least an order of magnitude is possible if a spectrofluorometric method of analysis is used.

July 27, 1976

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Anti-inflammatory activity of esters of acetic acid

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Phenylglycine n-heptyl ester has been found in both *in vitro* and *in vivo* tests to be a potent inhibitor of bradykinin, 5-hydroxytryptamine (5-HT), histamine and dextran in rats and of acetylcholine, histamine, 5-HT and anaphylaxis in guinea-pigs (Gecse, Zsilinszky & others, 1971). Recently, this compound and the corresponding ester of phenylalanine were shown to inhibit carrageenan and dextran responses in rat paws as well as arthritis induced by Freund's adjuvant in rats (Thomas & West, 1973). More recently, using a series of straight-chain esters of phenylglycine, Thomas & West (1974) reported that anti-inflammatory activity was possessed only by esters of phenylglycine with high molecular weight alcohols, the threshold being hexyl for dextran oedema and pentyl for carrageenan inflammation. Optimal activity in both types of inflammation resided in the heptyl ester.

We have now tested a series of straight-chain esters of acetic acid for their anti-inflammatory effects. Inflammation was induced by subcutaneous injections of carrageenan (1 mg in 0·1 ml normal saline) into the right hind-paws of groups of 6 Wistar rats (120–150 g) obtained from the Tuck colony. The increase in paw volume was recorded every hour for 5 h on a volume

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