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Note

Gas chromatographic assay of atropine in formulations containing atropine sulphate and cholinesterase reactivators

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Many organophosphates have been used extensively as agricultural insecticides, whilst others are potential nerve gases. Occupational exposure to the pesticidal organophosphates can occur during manufacture or use, and also poisoning may occur following oral ingestion for homicidal or suicidal reasons[1]. These compounds are potent anticholinesterase agents, and overdosage results in accumulation of acetylcholine. Injection of atropine is the standard treatment for organophosphate poisoning[2], but it has some deficiencies. It is ineffective against peripheral neuromuscular activity caused by the excess acetylcholine. Cholinesterase reactivators represent a significant advance in treatment of organophosphate poisoning, since they can reverse the symptoms not affected by atropine and act directly on the phosphorylated enzyme, resulting in hydrolysis and reactivation[3]. This leads to a more rapid return to normal levels of cholinesterase activity.

For effective therapy, it is necessary to use atropine to block the excess acetylcholine, together with a compound which will reverse the cholinesterase inhibition. Oximes are nucleophilic agents which have been shown to be effective as cholinesterase reactivators in treatment of organophosphate poisoning. Two of these compounds, pralidoxime [2-PAM-Cl, 2-(hydroxyiminomethyl-1-methylpyridinium) chloride] and toxogonin [obidoxime, bis-(4-hydroxyiminomethyl-1-pyridinomethyl ether) dichloride] have been used clinically in conjunction with atropine[4]. Combination of atropine sulphate with one of the oximes in a parenteral solution could provide a useful product for emergency treatment of organophosphate poisoning.

Compatibility and stability studies on pharmaceutical products require specific, sensitive assays. The conventional procedure for the oximes involves UV analysis, which has been shown to be suitable in the presence of atropine[5]. The latter compound has been assayed spectrophotometrically as the picrate salt[6]. However, problems were experienced when the method was used in accelerated stability studies on formulations containing atropine sulphate and oximes. Interference from breakdown products resulted in elevated readings at the optimum wavelength for determination of the atropine derivative. Several variations of the procedure are available, but none was found to be appropriate for determination of atropine in stored samples of these mixed formulations. Gas–liquid chromatographic (GLC) analysis using atropine analogues as internal standards[7] was examined as an alternative, but degradation of the oximes resulted in peaks which coincided with these reference compounds.

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A method has been developed for quantitative assay of atropine in the presence of cholinesterase-reactivating oximes and their degradation products. Mepyramine maleate was used as the internal standard. Problems associated with use of earlier analytical methods for the assay of mixed formulations of atropine sulphate and the oximes were eliminated.

EXPERIMENTAL

Apparatus

The instrument used was a Beckman GC-5 gas–liquid chromatograph with a flame ionization detector (Beckman Instruments, Fullerton, CA, U.S.A), connected to a Houston Omniscribe recorder (Fisher Scientific, Montreal, Canada). Separation was effected on a 1.83 m × 4 mm I.D. glass column, packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q (Pierce Chemical, Rockford, IL, U.S.A.). Column temperature was 190°C. Injection temperature was 255°C and the detector was at 285°C. Carrier gas was nitrogen, with a flow-rate of approximately 60 ml/min.

Chemicals

All reagents were analytical grade (Fisher Scientific). Pralidoxime (Ayerst Labs, Saint-Laurent, Canada), toxogonin (Astra Chemicals, Mississauga, Canada), atropine sulphate (BDH Chemicals, Poole, Great Britain) and the reference compound, mepyramine maleate (Poulenc, Montreal, Canada) were drug quality. Mepyramine maleate was prepated as an aqueous stock solution containing 5 μg/μl. Solutions of atropine sulphate for the calibration curve contained 0.14, 0.29, 0.57, 0.86 and 1.14 mg/ml.

Procedure

Solutions used in these assays contained up to 2 mg/ml atropine with varying concentrations of oximes up to 500 mg/ml. Representative samples were sealed in glass ampoules and subjected to an accelerated stability study at 90°C for 5 weeks. An aliquot of each product (0.5 ml) was placed in a 15 ml screw-top test tube with PTFE-lined cap. Mepyramine maleate solution (100 μl, 500 μg), ammonium hydroxide solution, 0.88 g/ml (2 ml), methylene chloride (10 ml) and ammonium sulphate ≈2 g, (sufficient to saturate) were added. The mixture was shaken for 10 min, then centrifuged for 5 min. The organic phase was transferred to clean tubes and the solvent evaporated using a stream of nitrogen. Each sample was reconstituted with methylene chloride (1 ml) and aliquots injected into the gas chromatograph. The peak height ratio for atropine/mepyramine maleate was calculated and the quantity of atropine sulphate present determined by reference to a calibration curve prepared previously. Concentrations of atropine sulphate used for the standard curve were in the range 0.14–1.14 mg/ml.

RESULTS AND DISCUSSION

The spectrophotometric assay for atropine involves formation of a picrate salt and measurement of the absorbance at 347 nm. The procedure gives reliable results

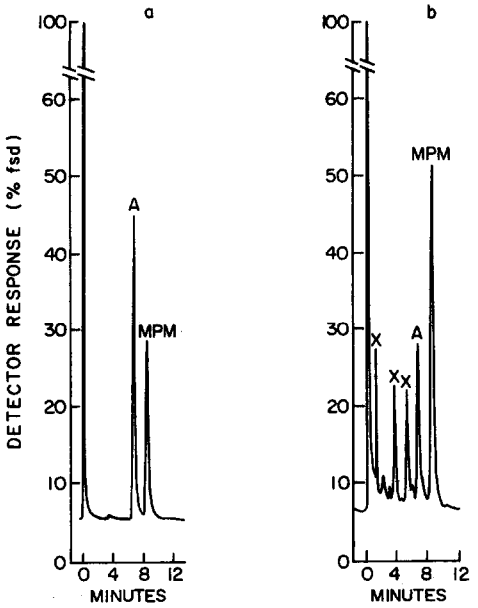

Fig. 1. (a) Chromatogram of freshly prepared solution of atropine sulphate, with mepyramine maleate standard. (b) Chromatogram of atropine sulphate, with mepyramine maleate standard in the presence of toxogonin breakdown products. fsd = Full scale deflection; MPM = mepyramine maleate; A = atropine sulphate; X = unidentified breakdown products of toxogonin.

with freshly prepared solutions containing both atropine sulphate and toxogonin, but accelerated stability studies at elevated temperatures result in breakdown of the toxogonin. The degradation products react with picric acid[8] giving a mixture which has significant absorbance at 347 nm. This method is not appropriate for the assay of atropine in the presence of degraded toxogonin.

GLC offers a reliable alternative for the quantitative analysis of atropine in mixtures containing oximes and their breakdown derivatives. The atropine analogues, homatropine and scopolamine, were found to be unsuitable as internal standards as they did not separate adequately from the components of the aqueous atropine sulphate–toxogonin solution which had been subjected to storage at 90°C for five weeks prior to extraction. Mepyramine maleate was selected as an internal standard on the basis of extraction properties and GLC retention time. A trace showing the separation of this compound from atropine, using a freshly prepared solution of atropine sulphate is shown in Fig. 1a. No interference from other compounds occurred, and when freshly prepared solutions of toxogonin were added, the atropine and mepyramine maleate were separated clearly from all other constituents of the mixture.

The recommended initial dose of toxogonin for treatment of organophosphate insecticide poisoning in man is 3–6 mg/kg[9], compared with a 2-mg dose of atropine[1]. It is clear that the quantity of oxime required is high relative to that of atropine, and any interference from the oxime or its degradation products would result in significant errors in the quantitation of atropine in solutions containing these components.

TABLE I

CALIBRATION CURVE FOR ATROPINE SULPHATE

mg/ml	*Mean peak height ratio atropine/mepyramine*	*C.V. (%)*
1.14	1.60	3.1
0.86	1.12	5.3
0.57	0.68	2.9
0.29	0.26	13.4
0.14	0.11	9.1

Slope = 1.50; y intercept = -0.15

Fig. 1b shows the separation obtained with a mixture of atropine sulphate and toxogonin which has been stored at 90°C for 5 weeks, prior to extraction and addition of the reference standard. The atropine and mepyramine maleate peaks were distinct and were free from interference by other components of the mixture. The method was equally effective when pralidoxime was substituted for toxogonin in the study.

The calibration curve for atropine sulphate is linear, as demonstrated in Table I. The data were produced using the peak height ratio method, with mepyramine maleate as the internal standard. The method was shown to be effective with mixtures containing oximes and their breakdown products. No significant difference was found between the results obtained for slope and intercept with atropine sulphate alone or in the presence of toxogonin or pralidoxime which had been degraded in an accelerated stability study at 90°C.

CONCLUSIONS

The spectrophotometric assay of atropine as its picrate salt has been shown to be unsatisfactory in the presence of partially degraded oximes. GLC provides a valid alternative procedure which can be used quantitatively with mepyramine maleate as an internal standard. This technique eliminates the problems of interference experienced with other analytical methods proposed for use in stability studies of solutions containing cholinesterase reactivators and atropine sulphate.

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