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Short Communication

Determination of ecothiopate iodide by ^{1}H-nuclear magnetic resonance spectroscopy

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Introduction

Ecothiopate iodide is a potent long-lasting cholinesterase inhibitor which is applied topically to the eye for the treatment of glaucomas and the diagnosis and treatment of accommodative esotropias [1]. Existing compendial methods for the assay of this drug as a raw material and powder for ophthalmic solution are based on an alkaline hydrolysis, followed by an iodometric titration of the liberated thiol under rigorous anaerobic conditions, and with a titrant solution that must be periodically restandardized owing to its instability [2]. A spectrophotometric method based on the change in absorbance at 226 nm has been used to monitor the rate of degradation of ecothiopate iodide in acid and alkaline media [3]. Whereas in acid medium the absorption spectra of ecothiopate iodide and (2-mercaptoethyl)trimethylammonium iodide, its hydrolytic product, are practically identical, in alkaline medium the salt form of the thiol shows a sufficiently higher molar absorptivity to make it distinguishable from the parent compound.

Ecothiopate iodide degrades in aqueous solution in a manner and to an extent that is dependent on the pH and temperature of the reaction medium. In alkaline medium it hydrolyses quantitatively at the S–P bond to yield (2-mercaptoethyl)trimethylammonium iodide and diethylphosphoric acid [3]. In acidic medium, degradation occurs almost exclusively by C–O fission of the alkyl side chain with the formation of one mole of ethanol for every mole of ecothiopate iodide lost [3].

The assay of ecothiopate iodide raw material and powder for ophthalmic solution can be accomplished with a minimum of reagents and procedural steps by the proton NMR spectroscopic method described here. An additional advantage to be gained by this method is the possibility of directly assessing the stability of the drug in aqueous solutions

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upon storage, after the commercial powder for ophthalmic solution has been reconstituted in the diluent provided.

Experimental

Apparatus

All ^{1}H-NMR spectra were obtained with a 90 MHz Varian EM-390 continuous wave spectrometer (Varian Instrument Group, Palo Alto, CA, USA) at an ambient probe temperature of 35°C and using a sweep time of 5 min.

Chemicals

Acetamide (98% minimum), deuterochloroform (C^2HCl_3, 99.5 atom % ^{2}H), and tetramethylsilane (TMS, 99.9+ %) were obtained from Aldrich Chemical Co., Milwaukee, WI, USA; deuterium oxide (^2H_2O, 99.7 atom % ^{2}H) and sodium 3-trimethylsilylpropionate-2,2,3,3-*d* (TSP-d_4) were obtained from Merck & Co. Inc., Rahway, NJ, USA. The sample of ecothiopate iodide was a gift from Ayerst Laboratories, Inc., Rouses Point, NY, USA. Vials of ecothiopate iodide for ophthalmic solution were obtained from commercial sources.

NMR assay

The contents of one vial (or the combined contents of several vials) of ecothiopate iodide for ophthalmic solution, equivalent to *ca* 62.5 mg of ecothiopate iodide, was mixed with *ca* 19.3 mg of acetamide, and the mixture was dissolved in 2 ml of chloroform with the aid of a vortex mixer. The clear solution was decanted into a 25 ml glass-stoppered flask, and evaporated to dryness under a stream of dry nitrogen gas. The residue was dissolved in 1–2 ml of ^2H_2O, and *ca* 0.5 ml of the solution was transferred to an analytical NMR tube that contained a few crystals of TSP-d_4. The NMR spectrum was recorded using a spin rate that produced no interfering side bands in the spectral region of interest (1.0–2.5 ppm). All chemical shifts were assigned with reference to TSP-d_4 taken as 0.00 ppm. After integrating the singlet at 2.01 ppm (acetamide) and the triplet at 1.38 ppm (ecothiopate iodide) at least five times, the average integral values were obtained. The quantity of ecothiopate iodide, in mg per vial, was calculated from:

$$\frac{A}{A'} \times \frac{EW}{EW'} \times \frac{C}{N},$$

where A was the average integral value for ecothiopate iodide, A' was the integral value for acetamide, EW was the equivalent weight of ecothiopate (molecular weight/6), EW' was the equivalent weight of acetamide (molecular weight/3), C was the weight, in mg, of acetamide taken for the assay, and N was the number of vials taken for the assay.

Results and Discussion

Besides being a satisfactory solvent for both the drug and the internal standard, chloroform also permitted the extraction of the dosage form without interference by potassium acetate, a common additive of freeze-dried ecothiopate iodide ophthalmic formulations. Obtaining the NMR spectrum in ^2H_2O solution permitted the differentiation of the resonance signals of the drug and internal standard from those produced by

degradates of ecothiopate iodide. An additional advantage provided by acetamide was the improved solubility of ecothiopate iodide in chloroform when acetamide was present.

Figures 1 and 2 are the 90 MHz ^{1}H-NMR spectra of ecothiopate iodide and acetamide in C^2HCl_3 and ^2H_2O, respectively. In C^2HCl_3 ecothiopate iodide displayed five resonance signals: a triplet centred at 1.41 ppm, originating from the 6 methyl protons of the ethyl side chains; a multiplet centred at 3.23 ppm, representing the 2 protons of the methylene group adjacent to sulphur; a singlet at 3.53 ppm, due to the 9 methyl protons attached to the quaternary nitrogen atom; a multiplet centred at 3.95 ppm, arising from the 2 protons of the methylene groups adjacent to the quaternary nitrogen atom; and a multiplet centred at 4.23 ppm, due to the 4 methylene protons of the ethyl side chains. In ^2H_2O solution the five resonance signals appeared at 1.38, 3,33, 3.21, 3.66 and 4.31 ppm, respectively. The resonance lines for the P–O–CH_2 and P–S–CH_2 protons underwent further splitting due to ^{31}P–^{1}H spin coupling (phosphorus nuclear spin = ½). The two multiplets arising from the N^+–CH_2 and N^+–C–CH_2–S protons deviated from the typical AA'XX' pattern of 1,2-disubstituted ethanes because of the additional coupling of these protons with ^{31}P, and to a lesser extent with ^{14}N (nuclear spin = 1). Further resonance signals detected in the NMR spectrum were found at 4.70 ppm (^2H_2O, Fig. 2), and at 2.91, 6.03, and 7.33 ppm (C^2HCl_3, Fig. 1), and are assigned to H^2HO, moisture, NH_2, and $CHCl_3$, respectively.

The quantitative analysis was based on the integration of the proton signals at 1.38 ppm for ecothiopate iodide and at 2.01 ppm for acetamide. The accuracy of the proposed method was verified by analysing a group of 15 synthetic mixtures containing the drug and internal standard, with the results presented in Table 1. The pooled mean ±s.d. was 99.9 ± 0.43% (c.v.:0.43%). The relative proportion of internal standard to

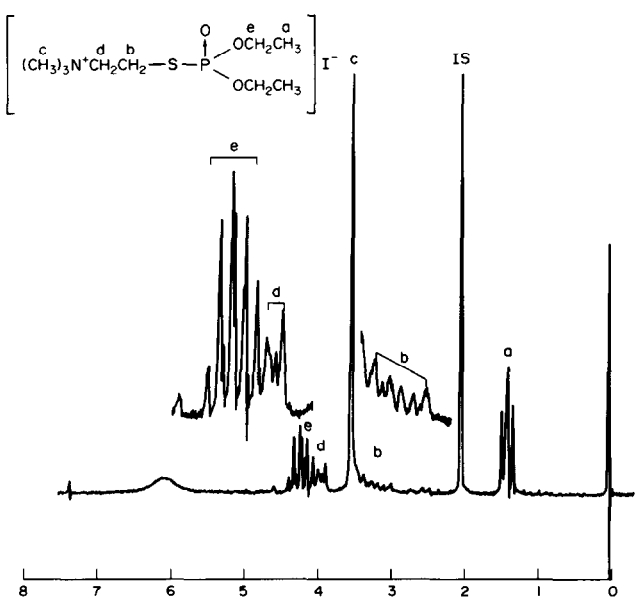

Figure 1
90 MHz ^{1}H-NMR spectrum of a mixture of ecothiopate iodide and acetamide, the internal standard, in C^2HCl_3 solution.

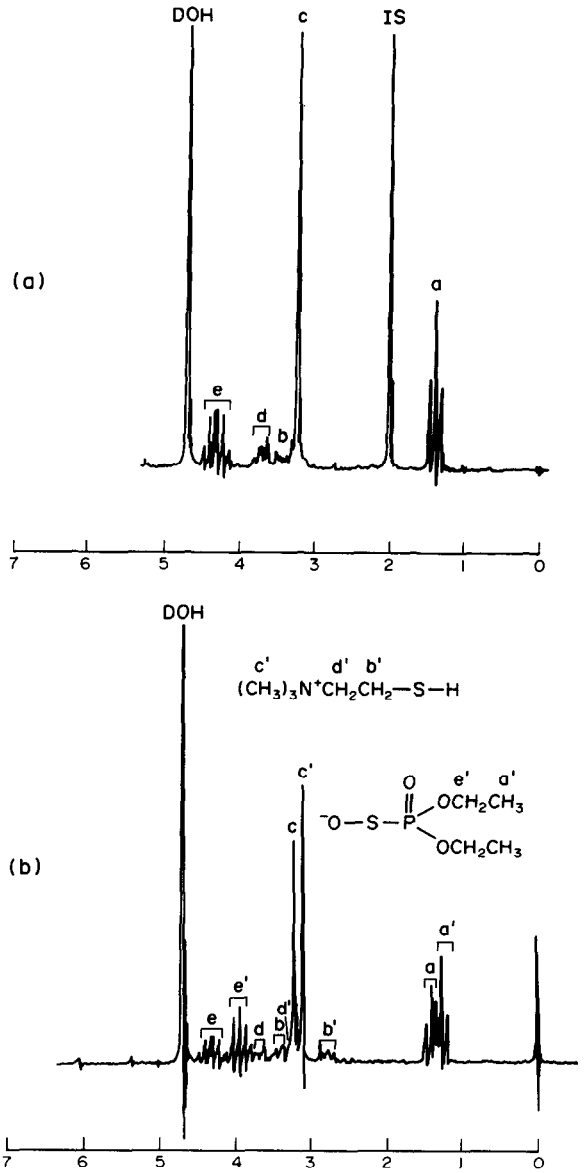

Figure 2
90 MHz ^{1}H-NMR spectra of (a) a mixture of ecothiopate iodide and acetamide, the internal standard, and (b) ecothiopate iodide and its alkaline hydrolysis degradation products in ^2H_2O solution.

drug had no significant bearing on the accuracy of the method for the range of concentrations shown in Table 1. Ecothiopate iodide remained unaltered in ^2H_2O solution. No significant differences were observed among recovery values obtained for solutions that were left standing at room temperature for periods of up to 7 h. Two strengths of commercial dosage forms (6.25 and 12.5 mg/vial) were analysed by the proposed method, and the assay results were found in all cases to be in good agreement with the declared quantities of ecothiopate iodide (Table 2). For 12.5 mg/vial samples the

Table 1
Recovery of ecothiopate iodide from standard mixtures*

Mixture no.	Acetamide added (mg)	Ecothiopate iodide		
		Added (mg)	Found (mg)	Recovery (%)
1	10.0	31.5	31.5	100.0
2	9.5	27.8	27.9	100.4
3	10.1	25.7	25.6	99.6
4	9.2	23.4	23.2	99.1
5	9.3	29.8	29.9	100.3
6	10.2	32.5	32.5	100.0
7	9.6	37.5	37.3	99.5
Mean				99.8
s.d.				0.47
c.v. (%)				0.47
8	9.7	32.5	32.4	99.7
9	9.9	31.0	31.0	100.0
10	9.4	31.8	31.7	99.7
11	9.3	27.2	27.3	100.4
12	9.5	27.9	28.0	100.4
13	9.9	28.6	28.5	99.7
14	9.8	33.4	33.2	99.4
15	9.0	39.2	39.4	100.5
Mean				100.0
s.d.				0.41
c.v. (%)				0.41

*Mixtures 1–7 were extracted as described for commercial dosage forms. Mixtures 8–15 were directly dissolved in the NMR solvent.

Table 2
^{1}H-NMR determination of ecothiopate iodide in commercial powders for ophthalmic solution

Manufacturer	Lot. no.	Amount declared (mg/vial)	Amount found	
			(mg/vial)	(%)
A	1	12.50	12.48	99.8
	2	12.50	12.51	100.1
	3	12.50	12.50	100.0
	4	12.50	12.47	99.8
	5	12.50	12.52	100.2
Mean				99.8
B	1	6.25	6.23	99.7
	2	6.25	6.25	100.0
	3	6.25	6.26	100.2
	4	6.25	6.25	100.0
	5	6.25	6.24	99.8
Mean				99.9

mean found was 99.8 (range 99.8–100.2, $n = 5$)% of declared, whereas the 6.25 mg/vial samples gave a mean of 99.9 (range 99.7–100.2, $n = 5$)% of declared.

Whereas ecothiopate iodide is fairly stable in neutral and moderately acidic aqueous media, it will readily hydrolyse to (2-mercaptoethyl)trimethylammonium iodide and diethylphosphoric acid in alkaline medium [3]. At room temperature or normal storage temperatures alkaline hydrolysis represents the main degradative pathway for ecothiopate iodide. In contrast, acid hydrolysis is only significant at high temperatures and therefore it is highly unlikely to occur under ordinary storage conditions. The study of the degradative behaviour of ecothiopate iodide previously required the combined use of iodometric titration, thin-layer chromatography, and ultraviolet spectrophotometry [3]. ^{1}H-NMR spectroscopy, on the other hand, offers a more direct and simpler means of detecting and determining the degradates of ecothiopate iodide. The presence of (2-mercaptoethyl)trimethylammonium iodide was suggested by the appearance of a singlet at 3.09 ppm and two multiplets centred at 2.77 and 3.23 ppm, due to the CH_2–S, $(CH_3)_3$–N^+, and N^+–CH_2 protons, respectively. The presence of diethylphosphoric acid was inferred from the appearance of a triplet at 1.27 ppm and a multiplet at 3.93 ppm, assigned to its methyl and methylene protons, respectively. These two degradates were easily formed in the laboratory by treating a solution of ecothiopate iodide in ^2H_2O with a trace of 40% NaO^2H in ^2H_2O. The ^{1}H-NMR spectrum of a mixture of ecothiopate iodide and its degradation products is shown in Fig. 2. Table 3 summarizes the ^{1}H-NMR assignments for the components of this mixture. In the event that degradation has taken place, the quantity of total ecothiopate iodide in the sample can be calculated from the integral value for the two overlapping triplets, one centred at 1.27 ppm and the other centred at 1.38 ppm (Fig. 2B), and the equivalent weight of ecothiopate iodide, i.e. the molecular weight normalized for the 6 absorbing protons at 1.38 ppm. The quantity of (2-mercaptoethyl)trimethylammonium iodide can be calculated from the formula used for calculating the quantity of ecothiopate iodide in commercial dosage forms except that one uses the integral value for the multiplet centred at 2.77 ppm and the equivalent weight for this degradation product, i.e. the molecular weight normalized for the 2 absorbing protons at 2.77 ppm. Hence, the quantity of intact ecothiopate iodide in the sample can be obtained as the difference between the value for the total drug and the value representing the degradate. In this manner the extent of degradation of ecothiopate was easily determined to a minimum of about 2% of the parent compound.

Table 3
^{1}H-NMR spectral assignments for ecothiopate iodide and its degradation products

		Chemical shift (ppm)				
		C^2HCl_3		^2H_2O		
Assignment	No. of protons	ETP	ETP	META	DEP	Multiplicity
C–CH_3	6	1.41	1.38	—	1.27	triplet
C–CH_2–S	2	3.23	3.33	2.77	—	multiplet
$(CH_3)N^+$	9	3.53	3.21	3.09	—	singlet
N^+–CH_2	2	3.95	3.66	3.23	—	multiplet
P–O–CH_2	4	4.23	4.31	—	3.93	multiplet

ETP = ecothiopate iodide; META = (2-mercaptoethyl)trimethylammonium iodide; DEP = diethylphosphoric acid.

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