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Short communication

Determination of atropine and obidoxime in automatic injection devices used as antidotes against nerve agent intoxication

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Abstract

A capillary gas–liquid chromatographic (GLC) and an ion-pair high performance liquid chromatographic (HPLC) method were developed for the assay of atropine sulphate and obidoxime chloride from a parenteral solution in commercial automatic injection devices. The injectors are aimed for the emergency treatment of poisoning by nerve agents. The two-step GLC method consists of extraction of atropine as a free base prior to GLC analysis using scopolamine as an internal standard. Obidoxime is determined directly in a diluted sample solution by reversed-phase HPLC using sodium 1-heptanesulphonate as a counter ion in the mobile phase. The relative standard deviation (R.S.D.) was 1.81% for the GLC procedure with injectors containing only atropine and 2.37% for the GLC of atropine in atropine–obidoxime injectors. The R.S.D. for the HPLC procedure of obidoxime in atropine–obidoxime injectors was 0.82%. The corresponding R.S.D.s for the sampling of atropine–obidoxime injectors were 0.36% and 0.27%. The coefficient of determination (r^2) was 1.000 for both methods. The recoveries at the target concentration averaged 101.0% and 98.7% with a standard error of the mean of 0.30 for both methods. The retention times for atropine and obidoxime were 6.27 and 4.29 min, respectively.

1. Introduction

The organophosphate nerve gas agents are a serious threat in the battlefield. These agents affect the nervous system by blocking the enzyme acetylcholinesterase, which plays an essential role in the process of transmitting information between nerves and from nerves to muscles and glands.

Atropine (AT) in combination with certain oximes, obidoxime (OB) or pralidoxime, in a parenteral solution is used for the emergency treatment of poisoning by toxic organophosphates. To be effective the therapy must begin within minutes after intoxication. With this in view, automatic injectors have been developed, which permit a rapid and convenient means for the intramuscular self-administration of the antidote [1–4].

In the battlefield the storage of pharmaceuticals is often complicated. Varying storage conditions may change, e.g., the given shelf-lives and stability prediction may become impossible. If preparations stored in such field conditions, are to be used, however, it is important to have a

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means to control and ensure their identity, strength, purity and quality [5–7].

Determinations of AT in pharmaceuticals have been performed by means of UV spectrophotometry [8], thin-layer chromatography (TLC) [9,10], high-performance liquid chromatography (HPLC) [11–23] and gas–liquid chromatography (GLC) [24–29]. GLC and HPLC are generally used today in the determination of AT. Few assays for the determination of OB have been reported. However, UV spectrophotometric [30], polarographic [31] and TLC [32] methods have been described. An HPLC [33] method was presented for blood samples. UV, TLC and polarographic methods for the determination of AT or OB are known to be associated with complicated and tedious sample preparations. Moreover, these methods are mostly aimed at one-component formulations [24,26]. Similarly, HPLC methods are suitable for the analysis of samples containing AT only [13–18]. Accordingly, investigations in our laboratory indicated that samples containing both AT and OB could not be analysed quantitatively by these methods. GLC procedures instead have been shown to distinguish well between AT and oximes, although interference by decomposition products from the latter compounds [26] and lack of sensitivity [17] have been experienced. In addition, because of unsatisfactory reproducibility [26], detection [26] or sample matrix [24,26,28] different to ours, the previous GLC methods described could not be applied as such in this study.

2. Experimental

2.1. GLC analysis of atropine sulphate

Equipment and supplies

The analyses were carried out on a Hewlett-Packard (Waldbronn, Germany) (HP) model 5890 gas chromatograph equipped with a flame ionization detector. ChemStation software was installed in a HP Vectra QS/20 personal computer (Hewlett-Packard, Roseville, CA, USA) for data handling. An HP-5 capillary cross-linked 5% phenylsilicone column (Hewlett-Packard, Avondale, PA, USA), 25 m × 0.32 mm I.D. with a film thickness of 0.52 μm was used. The column was operated in the split mode with a splitting ratio of 10:1. The column temperature was programmed as follows: initial temperature 160°C, increased at 20°C min^{-1} to 200°C, then at 50°C min^{-1} to 270°C, the final temperature being held for 4.20 min; the total time was 7.60 min. The injection port was maintained at 255°C and the detector at 285°C. The carrier gas (helium) flow-rate was 1.0 ml min^{-1}; the hydrogen and air flow-rates were 30 and 400 ml min^{-1}, respectively. Under these conditions the retention times of AT and the internal standard (I.S.) scopolamine (SC) were 6.27 and 7.23 min, respectively (see Fig. 1). The least-squares linear regression equation for AT was $y = 2.0397x - 0.0058$, where y is the AT/SC peak-area ratio and x is the AT concentration in mg ml^{-1}. The calibration graph for AT was calculated using Harward Graphics software (Software Publishing, Mountain View, CA, USA).

Reagents

Atropine (*dl*-hyoscyamine) sulphate monohydrate (Sigma, St. Louis, MO, USA) and scopolamine hydrobromide trihydrate pure substances (Boehringer Ingelheim International, Ingelheim am Rhein, Germany) were used as references. Chloroform (99.4%) (Merck, Darmstadt, Germany) was of analytical-reagent grade.

The composition of AtroPen (Solvay Duphar, Weesp, Netherlands) was atropine sulphate (2.86 mg), glycerin (17.81 mg), citric acid (4.67 mg), sodium citrate (4.35 mg) and phenol (4.00 mg) in water (1 ml). The composition of ComboPen (Solvay Duphar) was atropine sulphate (1.00 mg), obidoxime chloride (87.50 mg) and phenol (4.20 mg) in water (1 ml).

Borax buffer (pH 9.80) was prepared by dissolving borax (4.77 g) and sodium hydroxide (0.40 g) in deionized water (100 ml) and adjusting the pH to 9.80 by adding sodium hydroxide.

Sample preparation procedure

Assay for AtroPen samples. A 500-μl volume of I.S. solution (SC, 1.00 mg ml^{-1}) was added to

250 μl of sample solution. The solution was made alkaline with 140 μl of ammonia solution (1.2%) and 4.0 ml of buffer solution (pH 9.80) were added. The mixture was extracted with 4 ml of chloroform and the organic layer was transferred into a vial. The chloroform phase was evaporated to dryness on a water-bath (70°C). The dried residue was dissolved in 1.00 ml of chloroform and 1 μl was injected into the GC column.

Assay for ComboPen samples. A 500-μl volume of I.S. solution (SC, 1.00 mg ml^{-1}) was added to 500 μl of sample solution. The solution was made alkaline with 330 μl of ammonia solution (6.3%) and 4.0 ml of buffer solution (pH 9.80) were added. The procedure was continued as described for AtroPen samples.

2.2. HPLC analysis of obidoxime chloride

Equipment and supplies

The assay was developed using an HP 1050 series liquid chromatograph (Hewlett-Packard) equipped with a high-pressure quaternary pump, variable-wavelength detector and a 20-μl loop injector. An HP 3396A series integrator was obtained from Hewlett-Packard. The stationary phase in the RP-18 column (Merck), 125 mm × 4 mm I.D., was LiChrospher 100 with an average particle diameter of 5 μm. The mobile phase consisted of 16% acetonitrile and 15% methanol in water containing sodium 1-heptanesulphonate (10 m*M*) as a counter ion. The pH of the eluent was 6.30. The flow-rate of the mobile phase was 1.5 ml min^{-1} and the absorbance was measured at 220 nm. All separations were performed at ambient temperature. A 500-μl volume of comboPen sample was diluted to 25.00 ml and 100 μl were injected into the 20-μl loop injector. The retention times were 2.40 and 4.29 min for phenol and OB chloride, respectively (see Fig. 2). OB was determined by the external standard method. The calibration graph for OB was calculated as for AT. The linear regression equation was $y = 92.6972x + 1.9171$, where y is the peak area at 220 nm expressed in integration units and x is the concentration of OB chloride in mg ml^{-1}.

Reagents

Acetonitrile (99.8%) and methanol (99.9%) (Mallinckrodt Specialty Chemicals, Paris, KY, USA) were of HPLC-grade. Sodium 1-heptanesulphonate (98%) (Sigma, St. Louis, MO, USA) was of analytical-reagent grade. Obidoxime chloride pure substance used as a reference was obtained from Duphar (Amsterdam, Netherlands).

3. Results and discussion

In the present study the simultaneous determination of AT and OB by HPLC was unsuccessful under both isocratic and gradient conditions. Either retention of OB was irreversible under the conditions defined for AT or AT was not quantitatively eluted under the conditions defined for OB. The determination of AT and OB simultaneously by GLC would have required a tedious and time-consuming sample preparation procedure owing to their different extraction properties. In addition, OB has proved to be a relatively unstable compound at the elevated temperatures needed for GLC analyses [26]. Hence it seemed reasonable to develop separate methods for the determination of AT and OB in the auto-injectors: a GLC method for atropine sulphate and an HPLC method for obidoxime chloride.

In the GLC method proposed here, the sample was made alkaline before treatment with chloroform in order to extract AT quantitatively as a free base. Preliminary studies indicated that the recovery of extracted AT was optimum at pH 9.80, to which the pH of the sample solutions was adjusted with borax buffer. SC was selected to be the I.S. on the basis of its extraction properties and GLC retention time. Compound decomposition caused by initial temperatures higher than 160°C and programming rates faster than 20°C min^{-1} was avoided by using a two-step temperature programme. This also provided good separation and a short analysis time.

The calibration graph was constructed from analyses of standard solutions containing known concentrations of AT sulphate. The graph was found to be linear over the concentration range

0–1.50 mg ml^{-1} (r^2 = 1.000). The analytical reproducibility for the entire two-step GLC procedure was evaluated by analysing six successive samples of a single auto-injector. The relative standard deviations (R.S.D.s) were 1.81% for AtroPen and 2.37% for ComboPen samples. The reproducibility of sampling determined for ComboPen samples was 0.36%.The recoveries of AT were determined by assaying standard samples using a standard addition method. The average recovery of AT at the target concentration (0.50 mg ml^{-1}; n = 6) was found to be 101.0% with a standard error of the mean (SEM) of 0.27%.

A GLC trace of an AtroPen sample is presented in Fig. 1. The peaks are symmetrical and the retention times are sufficient for the distinct separation of AT and SC. OB together with the pharmaceutical additives in auto-injector samples did not interfere with the determination of AT because they were not extracted with AT. The AT/SC peak-area ratio was calculated and the amount of AT sulphate present in the sample was determined with reference to the calibration graph.

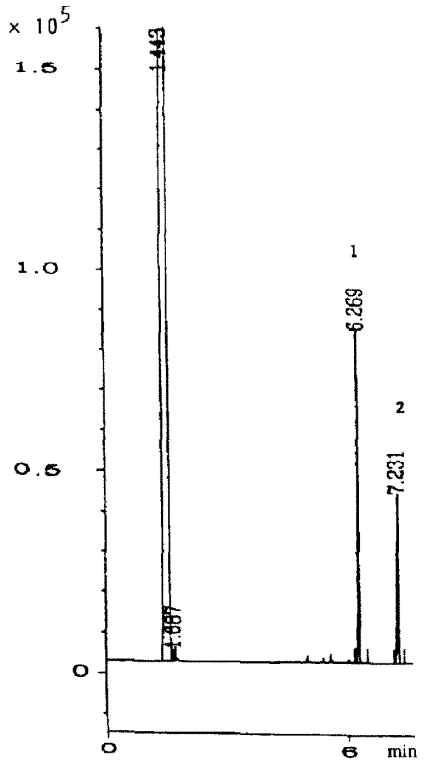

Fig. 1. GLC of an AtroPen auto-injector sample. Peaks: 1 = atropine; 2 = scopolamine (internal standard).

Because of the quaternary structure of pyridinium aldoximes ion-pair reversed-phase LC was expected to be a suitable method for determination of OB in ComboPen samples. In this study sodium 1-heptanesulphonate proved to be the most appropriate ion-pairing agent to form a lipophilic complex with OB.

The calibration graph obtained with spiked standard solutions of OB was found to be linear over the concentration range 0–2.50 mg ml^{-1} (r^2 = 1.000). The analytical reproducibility for OB was determined by analysing six successive sample solutions diluted from a single ComboPen injector. The R.S.D. was 0.82%. The reproducibility of sampling was 0.27%. As the reproducibility was satisfactorily controlled by the loop injector, an internal standard was not required. The recovery of the HPLC method determined by assaying six spiked standard solutions of OB chloride at the target concentration (1.75 mg ml^{-1}) averaged 98.7% with an SEM of 0.30%.

A chromatogram of a ComboPen sample is presented in Fig. 2. It is seen that phenol produced a separate peak, which did not interfere with the determination of OB. Tailing of the OB chloride peak might have been caused by injecting a relatively large amount of drug (35 μg).

Conclusions

The described GLC and HPLC methods are accurate and precise for the determination of atropine sulphate and obidoxime chloride in parenteral auto-injector solutions. The linearity and reproducibility are very good for both methods. The recoveries were 101% and 99%, respectively. The methods are also rapid and fairly simple. The GLC method consists of two steps: extraction of atropine as a free base with chloroform and subsequent GLC analysis. Obidoxime

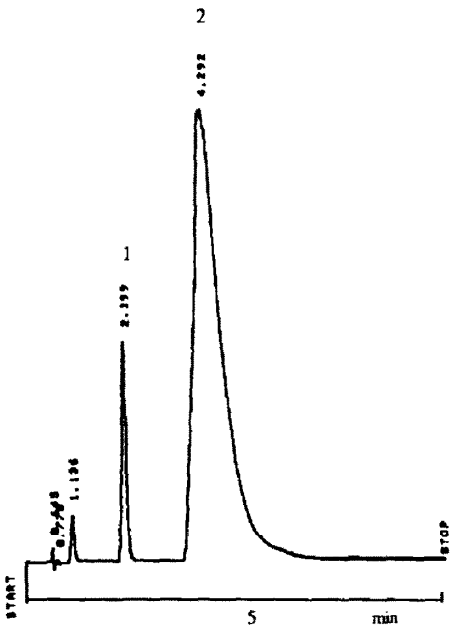

Fig. 2. HPLC of a ComboPen auto-injector sample. Peaks: 1 = phenol; 2 = obidoxime chloride.

is determined directly in diluted sample solution by ion-pair HPLC. The methods can be readily adapted to, e.g., stability studies of AtroPen and ComboPen auto-injectors.

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