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Journal of Chromatography A, 1057 (2004) 237–239
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Simple high-performance liquid chromatographic method for determination of atropine and obidoxime in a parenteral injection device

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Received 27 August 2004; received in revised form 2 September 2004; accepted 14 September 2004

Abstract

Atropine and obidoxime in a parenteral injection device are determined by simple HPLC method simultaneously without any pretreatment at 228 nm. The relative standard deviations (R.S.D.) were below 1.6% for the compounds. The correlation coefficient was greater than 0.999 for both compounds in the calibration range. The recoveries at 5 mg/L concentration averaged as 95% for atropine and 102% for obidoxime. The uncertainty of the measurements for atropine and obidoxime was 2.8% and 2.4%, respectively.

Keywords: Atropine; Obidoxime; Method validation; Uncertainty of the method

1. Introduction

The organophosphate nerve gas agents are a serious threat in the battlefield. The therapy against these gases must begin within minutes after intoxication. Parenteral injection devices (PIDs) have been developed for rapid and convenient solution to this problem. Atropine in combination with certain oximes in a parenteral solution is used for the emergency treatment of poisoning by toxic organophosphates. Atropine is suitable to counteract the muscarinic effects of the ensuing cholinergic crisis [1]. However, it is ineffective at nicotinic sites [2]. To improve neuromuscular function and to diminish respiratory insufficiency; antidotes reactivating inhibited acetylcholinesterase (AchE) have been developed [1]. As an antidote mostly obidoxime [*N*,*N*′-oxy-dimethylene bis(pyridinium-4-aldoxime) dichloride] is used in several European countries [3].

The storage of these pharmaceuticals is often complicated. The given shelf-lives and stability prediction may become impossible. It is important to control their identity and quality. Most techniques used in the literature are developed to determine only one component [3–7]. GLC-FID [4,7] was preferred technique for the determination of atropine. Reversed-phase HPLC [3,5,6] and ion-pair HPLC were the applied techniques in the determination of obidoxime. We have found few methods that can be applied to directly to both of atropine obidoxime derivatives in PID [8,9]. Ion-pair HPLC [8] and reversed-phase HPLC techniques [9] were developed in order to determine both compounds in one run at a single wavelength using UV detection.

For the first time, an attempt was made to develop a relatively simple method to separate and quantify the obidoxime and atropine in PIDs that does not involve a prior separation of the solution components, which are measured at a single wavelength and using only silica gel column.

2. Experimental

2.1. Chemicals

Atropine sulfate monohydrate (99–101%) was purchased from Merck. Obidoxime chloride (99.8%) was obtained

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doi:10.1016/j.chroma.2004.09.048

from Salubris Inc., USA. Analyzed PIDs were supplied from DIOMED Company, Istanbul. Acetonitrile (99.9% HPLC grade), *ortho*-phosphoric acid (99.999%) and sodium dihydrogen phosphate (99%) were purchased from Aldrich Company.

2.2. Apparatus

HPLC analyses were carried out on a Shimadzu VP series (Tokyo, Japan) comprising a LC 10AD pump, SIL 10AF auto injector with 50 μL sample loop, SPD 10AV UV detector and Class VP data module.

2.3. Chromatographic conditions

The chromatographic separations were performed, using a Nucleosil 100-5 (Macherey-Nagel) silica gel (25 cm × 4.6 cm i.d.). The mobile phase selected for the method validation and for the determination of the atropine and obidoxime chloride was acetonitrile–phosphate buffer (40:60, v/v). The phosphate buffer was prepared with 3.25 mM sodium dihydrogenphosphate and concentrated *ortho*-phosphoric acid to adjust the pH to 3.5. The prepared phosphate buffer was filtered through a 0.45 μm Millipore Millex-HV filter. Before use, the mobile phase was degassed for 15 min in an ultrasonic bath. The samples were monitored with UV detection at 228 nm at the flow rate of 1 mL/min. The injection volume was 10 μL for all samples. The sample cooler temperature and the column temperature were kept at 15 °C and 40 °C, respectively.

2.4. Preparation of standard stock and test solutions

Ten mg of atropine sulfate and obidoxime chloride were weighed and transferred to 10 mL volumetric flask. It was diluted with mobile phase (acetonitrile–phosphate buffer, 40:60, v/v). The working solutions were prepared from the stock solutions by diluting with mobile phase.

The analyzed PIDs contain 2 mg atropine sulfate and 220 mg obidoxime chloride dissolved in 2 mL water. By pressing the top of the PID, the mixture was released. This solution was diluted to 10 mL with the mobile phase. Five mL of this solution was again transferred to 10 mL volumetric flask and diluted with the mobile phase and lastly 1 mL of aliquot taken from the flask was diluted to 10 mL with the mobile phase to obtain 10 mg/L of atropine and 1100 mg/L of obidoxime.

3. Results and discussion

3.1. Detection limits, limits of quantification, linearity, precision, recovery and uncertainty

The limits of detection (LODs) of the proposed method were found as 1.2 mg/L for atropine and 0.4 mg/L for obidoxime determined by considering a value, which was three times the signal to background noise (S/N) ratio using 10 mg/L of atropine and 50 mg/L of obidoxime solutions. The limits of quantification (LOQs), which were 4 mg/L and 1.2 mg/L for atropine and obidoxime, respectively (10 times the S/N for the above concentrations).

The linearity of the method was assayed by analyzing standard solutions in the range of 5–500 mg/L for atropine and 100–1000 mg/L for obidoxime. Correlation coefficients (r) were ≥ 0.999. The linear regression equation was $y = 2250x + 4655$ for atropine and $y = 11408x + 198739$ for obidoxime, where y is the peak area and x is the concentration in mg/L.

Precision of the method was determined by repeating the measurement three times at four-concentration level (100, 250, 500 and 1000 mg/L) for atropine and obidoxime on the same day. The R.S.D. values were found to be below 2% for atropine and 0.3% for obidoxime indicating good repeatability. The R.S.D. values according to the retention time at the same day at each concentration level changed between 0.1% and 1.6%, indicating again very good repeatability.

Recovery experiments were conducted to determine the accuracy of the method for the quantification of obidoxime and atropine. Recovery of the method was checked at around quantification limit values for atropine and obidoxime in triplicate. Recoveries were 95% (5.0 mg/L) for atropine and 102% (5.0 mg/L) for obidoxime.

Sources and quantification of the uncertainty for the applied method were determined by using EURACHEM/CITAC Guide, 2000 [10]. For both of the analytes, the maximum contribution comes from the calibration curves of the atropine and obidoxime. The percent relative uncertainties of atropine

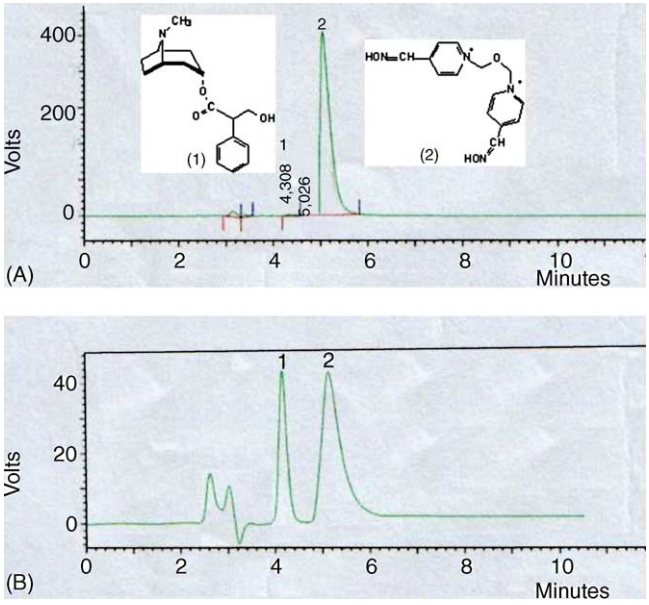

Fig. 1. (A) A representative chromatogram of analyzed PID solution (10 mg/L atropine, 1100 mg/L obidoxime). (B) A chromatogram of 100 mg/L of atropine and obidoxime standard solution.

and obidoxime were 2.8% and 2.4%, respectively, at 95% confidence level ($k = 2$) for 500 mg/L sample solutions.

3.2. Application of the method

The method developed has been applied successfully for routine analysis of atropine and obidoxime in PIDs.

A chromatogram of an analyzed PID is presented in Fig. 1. It was seen that atropine and obidoxime gave well-resolved peaks. Atropine itself gave a maximum signal at 203 nm and obidoxime gave its maximum signal at 280 nm. However, they both showed an absorbance at 228 nm. This will create a great advantage if working at a single wavelength is the only choice in a UV detection system.

4. Conclusions

A simple HPLC method has been developed and validated for the simultaneous determination of atropine and obidoxime in PIDs using only silica column at 228 nm. The method is simple, precise and accurate and it is useful for the routine determination of stability tests of PIDs in the formulation during storage within 6 min.

Acknowledgements

The authors of this paper would like to thank Dr. Tanıl Kocagöz for supporting this research financially.

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