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Liquid Chromatographic and Potentiometric Methods for the Determination of Oxomemazine in Pharmaceutical Preparations

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Abstract

Simple and selective two different techniques are developed for the determination of oxomemazine in pharmaceutical preparations. A rapid (less than 6 min) and accurate high performance liquid chromatography(HPLC) method has been developed where chromatographic analysis is performed on Nova-Pak® C_{18} column (3.9 mm × 150 mm, 5 µm) with an ammonium formate buffer adjusted with formic acid to pH 3.0 and acetonitrile (75: 25, v/v) as a mobile phase, and detection at 235 nm. Good linearity (0.99, r^2), accuracy (≥ 99.20%), and precision (≤ 0.6 RSD) were obtained. Potentiometric measurements are based on tetrakis (*p*-chlorophenyl) borate-oxomemazine ion-pair complex as an electroactive species incorporating in a plasticized poly vinyl chloride (PVC) membrane with *o*-nitrophenyl octyl ether (*o*-NPOE) or dioctyl phthalate (DOP). The sensor exhibits fast and stable Nernstian response for oxomemazine over the concentration range of $1.0 \times 10^{-5} - 1.0 \times 10^{-2}$ mol L^{-1} and pH range of 3.5 - 6.0. The sensor shows reasonable selectivity towards oxomemazine hydrochloride over many cations. No significant interferences are caused by drug excipients and diluents. Results with an average recovery of 100.6% and a mean standard deviation of 0.77% of the nominal were obtained.

Keywords

HPLC, Potentiometry, Oxomemazine Hydrochloride, Pharmaceutical Preparations

1. Introduction

Oxomemazine, a phenothiazine derivative, is an antihistamine used for the symptomatic relief of hypersensitivity reaction. It is also an ingredient of compound preparations for the symptomatic treatment of coughs and the common cold. It is given orally in doses equivalent to 10 to 40 mg of oxomemazine daily. Oxomemazine may also be administered rectally in form of suppositories. Oxomemazine hydrochloride (OXO-HCl) has been used similarly by mouth. It is chemically known as N,N,β-trimethyl-3-(10H-phenothiazin-10-yl)-propan-1-amine-5,5-dioxide [1] (Figure 1).

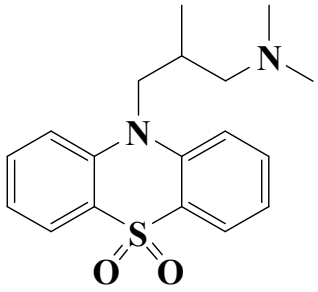

Figure 1. *Chemical structure of oxomemazine.*

The assay of the drug in pure and dosage forms is, as far as we know, not official in any pharmacopoeia, and therefore requires much more investigation. The different analytical methods that have been reported for its determination include HPLC [2, 3], gas chromatography (GC) [4, 5], thin layer chromatography (TLC) [6] and liquid chromatography–electrospray ionization-mass spectrometry (LC-ESI-MS) [7]. In the literature, only few spectrophotometric [8-12], infra red (IR) [13, 14], microcolorimetric titrations [15] have been reported. Volumetric titrations [16] and capillary electrophoresis [17, 18] have also been described. Focus of the present study was to develop an accurate, precise and robust liquid chromatographic method for the determination of oxomemazine hydrochloride in tablets. The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [19]. On the other hand, there is only one potentiometric method [20] based on sensor for the determination of oxomemazine has been reported in the literature so far, therefore we have developed a new potentiometric sensor based on tetrakis (*p*-chlorophenyl) borate-oxomemazine ion- pair complex as a novel electroactive species incorporating in a poly (vinyl chloride) matrix membrane plasticized with either *o*-nitrophenyl octyl ether or dioctyl phthalate.

2. Materials and Methods

Materials

Pure OXO-HCl was purchased from Amriya for Industries, Alexandria, Egypt. HPLC grade acetonitrile was obtained from SDS (de Valdonne, France). Analytical grade ammonium formate was purchased from Sigma-Aldrich, Germany. Formic acid was obtained from E. Merck (Darmstadt, Germany). Toplexile syrup (0.033 g OXO-HCl / 100 mL) was purchased from the market. Grade 1 water was obtained from a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA). Potassium tetrakis (*p*-chlorophenyl) borate (PT*p*-ClPB) (Figure 2) and tetrahydrofuran (THF) were obtained from Aldrich Chemical Co. (Milwaukee, USA). PVC (Breon S 110/0P) was obtained from BP Chemicals International (Barry, UK), *o*-nitrophenyl octyl ether (*o*-NPOE) was purchased from Fluka (Buchs, Switzerland) and dioctyl phthalate (DOP) from BDH (Poole, England).

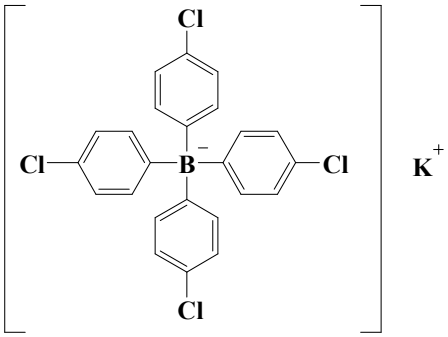

Figure 2. *Chemical structure of potassium tetrakis(p-chlorophenyl)borate.*

HPLC method

Instrumentation

The LC system consisted of a Waters model 481 UV detector, a Shimadzu LC-6A pump, and a model 7125 injector (Rheodyne, Berkeley California, USA) with 20 µL sample loop. The output signals were monitored and integrated using Perkin-Elmer TotalChrom software (version 6.2.1.).

Chromatographic conditions

The elution was isocratic. The mobile phase consisted of a mixture of aqueous 0.05 M ammonium formate adjusted with formic acid to pH 3.0 and acetonitrile (75: 25, v/v). Ammonium formate buffer of pH 3.0 (0.05 mol L^{-1}) was prepared as follows: [21] a 3.15 g of ammonium formate was dissolved in a 950 mL of water, pH was adjusted to value 3.0 ± 0.1 with formic acid (diluted with water in ratio 1:5), and buffer was diluted to 1000 mL with water and then was filtered through a 0.45-µm (HVLP, Germany) membrane filter. The mobile phase also was filtered through a 0.45-µm (HVLP) filter prior to use. A symmetry C_{18} analytical column (3.9 mm × 150 mm, 5 µm particle size) (Waters, USA) was used for determination of the drug under investigation. The flow rate of mobile phase was a 1.0 mL min^{-1} and the column was operated at ambient temperature (~25°C). Sample injection volume was a 20 µL and UV detector was set at a wavelength of 235 nm.

Preparation of stock and working standard solutions

The stock solution of OXO-HCl (500 µg mL^{-1}) was prepared by dissolving a 0.05 g of OXO-HCl (99.98%) in methanol in a 100-mL volumetric flask. Six different working/calibration solutions in mobile phase were prepared by appropriate dilution of the stock solution. The concentration range of the working standard solutions was 0.1-100.0 µg mL^{-1} as shown in the calibration curve (Figure 3).

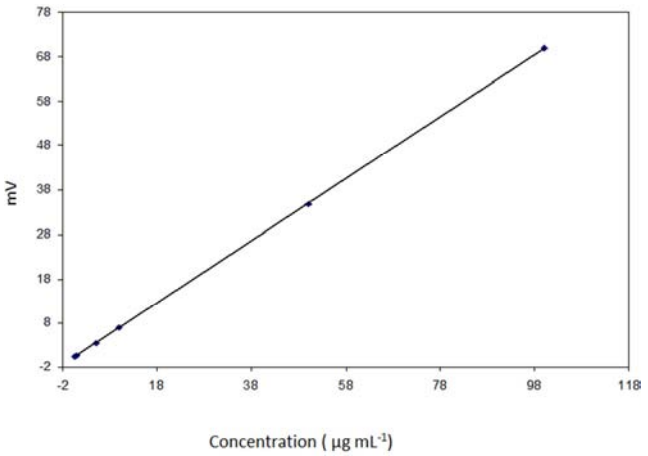

Figure 3. *The calibration curve of (OXO-HCl).*

Sample preparation

Each 100 mL syrup contains 0.033 g of (OXO-HCl). 1 mL of the syrup was dissolved in different volumes (10, 25, and 50 mL) of methanol to give different concentrations (33, 13.2, and 6.6 µg mL^{-1}) respectively. A typical chromatogram of (OXO-HCl) is shown in (Figure 4).

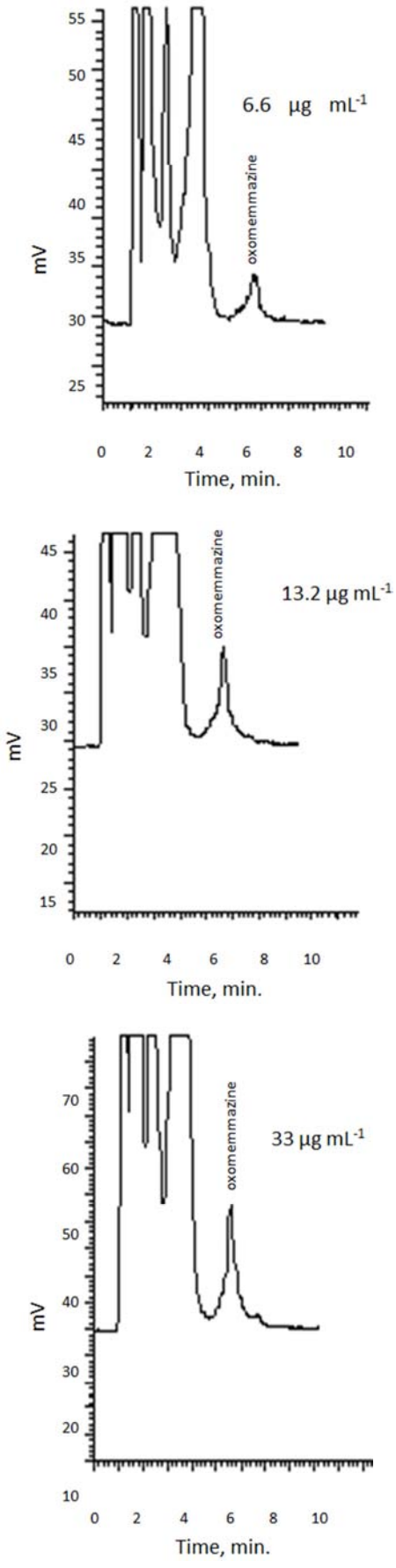

Figure 4. *The chromatograms of different concentrations of (OXO-HCl).*

Potentiometric method

Instrumentation

Electrochemical measurements were made at room temperature (25±1°C) with a PTI-15 digital pH meter by using oxomemazine membrane sensor in conjunction with an EIL type RJ 23 calomel reference electrode. A glass Ag-AgCl combination electrode (consort, S 210 B BB5) was used for pH measurements.

Oxomemazine sensor

Oxomemazine membrane sensor was prepared and assembled as described previously,[22] where the constitution of the sensor was as follows: potassium tetrakis(*p*-chlorophenyl)borate (PT*p*-ClPB) (40 mg) (7.02 mass%), *o*-nitophenyl octyl ether or dioctyl phthalate(360 mg) (63.16 mass%) and PVC (170 mg) (29.82 mass%). The internal reference electrode was a Ag-Ag chloride wire (~0.5 mm) immersed in a 1×10^{-1} mol L^{-1} oxomemazine inner filling solution. The sensor was conditioned by soaking in 1×10^{-1} mol L^{-1} oxomemazine solution for at least two days before use and was stored in the same solution when not in use.

Sensor calibration

The sensor was calibrated by spiking with successive aliquots of standard solution into a 1.0×10^{-5} mol L^{-1} solution of the calibrant. The electromotive force (e.m.f.) values were recorded and plotted as a function of the logarithm of the oxomemazine concentration. The calibration graph was used for subsequent determination of unknown concentrations of oxomemazine.

Selectivity coefficients

The potentiometric selectivity coefficients $\left(k_{OXO,B}^{pot}\right)$ were evaluated under the static mode of operation by the separate solution method [23, 24] where the potential of a cell comprising the OXO sensor and a reference electrode was measured with two separate solutions of OXO and interfering ions with the same activity (1×10^{-2} mol L^{-1}). E_{OXO} and E_B were the measured potential values for OXO and the interfering ions, respectively. The value of $\left(k_{OXO,B}^{pot}\right)$ was calculated from the equation

$$\log\left(k_{OXO,B}^{pot}\right) = \frac{\left(E_B - E_{OXO}\right)}{S} + \left(1 - \frac{Z_{OXO}}{Z_B}\right)\log a_{OXO}, \quad (1)$$

where S is the slope of the calibration plot and $a_{oxo.}$ the activity of oxomemazine hydrchloride ions; $Z_{oxo.}$ and Z_B the charges of the OXO and interfering ions, respectively.

Determination of oxomemazine in pharmaceutical preparations

A 11.6 mL aliquot (equivalent to 3.8 mg) of Toplaxile drug (0.033 g /100 mL) was quantitatively transferred to a 50-ml volumetric flask then diluted to the mark with doubly distilled deionized water. The pH was adjusted to (4.5) then the potential of the sensor was measured and compared with the calibration graph.

3. Results and Discussion

HPLC method

Method development

Choice of stationary phase

Chromatographic method was used on two batches of C_{18} columns provided by Waters (3.9 mm × 150 mm, 5 µm) and another manufacture to check the column performance. Due to difference in peak shape and in the retention time, the waters column gave the best results.

Choice of mobile phase

(OXO-HCl) was eluted before 6 min with a mobile phase composed of an ammonium formate buffer of pH value of 3.0 and acetonitrile (75: 25, v/v) but by using a mobile phase composed of ammonium formate and acetonitrile (60: 40, v/v) adjusted at the same pH value, (OXO-HCl) was eluted at about 2 min. However, at the same composition and different pH, no significant differences in retention time were observed. Hence a mobile phase consisting of ammonium formate and acetonitrile (75: 25, v/v) adjusted at pH value of 3.0 was chosen.

Method validation

Only (OXO-HCl) was observed by analyzing the syrup solution samples, showing that the method was selective.

Limits of detection and quantitation

The limit of quantitation (LOQ) was established at a signal-to-noise ratio of 10. The LOQ of (OXO-HCl) was determined by six injections of drug at the LOQ concentration and was found to be 0.3 µg mL^{-1}.

The limit of detection (LOD) was calculated from the equation

$$\mathrm{LOD} = \frac{3.3.\sigma}{S}$$

where σ is noise peak-to-peak of baseline in the chromatogram of placebo solution, S is slope of the regression line acquired by measuring of the linearity. The limit of detection (LOD) of (OXO-HCl) was 0.1 µg mL^{-1}.

Linearity

Linearity was evaluated by analysis of working standard solutions of (OXO-HCl) at six different concentrations [19]. Signals and concentrations of the drug were subjected to regression analysis to calculate the calibration equation and correlation coefficients. The regression equation obtained for the pharmaceutical preparations was y = 0.6998x - 0.0477 (r = 0.9993, n = 6). The range of linearity was 0.1 – 100.0 µg mL^{-1}.

Accuracy

Accuracy of the method was determined by analyzing three different solutions made from 1 mL of syrup. The solutions of theoretical concentration levels at 6.6, 13.2, 33 µgmL^{-1} were prepared in triplicate and analyzed for 3 consecutive days. Solutions for the calibration curves were prepared fresh every day. The assay accuracy variation shown in terms of relative mean error (RME) and% recovery are tabulated in Table 1 [19]. The RME values are below ±1.0% for the intra-day assay experiments. Due to the good accuracy, no internal standard was needed.

Precision

Table 1. *Accuracy of determination of (OXO-HCl) in syrup by using HPLC.*

Day of analysis	Theoretical concentrations (µg mL^{-1})	Actual concentrations (µg mL^{-1}) (n=3)	Recovery %	RME %
Day 1	6.6	6.56	99.40	-0.61
	13.2	13.15	99.62	-0.38
	33.0	33.06	100.20	0.18
Day 2	6.6	6.55	99.20	-0.76
	13.2	13.15	99.62	-0.38
	33.0	33.05	100.2	0.15
Day 3	6.6	6.55	99.20	-0.76
	13.2	13.13	99.46	-0.53
	33.0	32.95	99.84	-0.15

Precision of the method for the determination of (OXO-HCl) was studied using the parameters repeatability, intermediate precision, and robustness.

Repeatability in the intra-day variations in assay obtained at different concentration levels is expressed in terms of RSD values calculated from the data of each day for 3 days. RSD values of assay were found to be below 0.5% (Table 2).

Table 2. *Inter- and Intra-day assay variation of (OXO-HCl) using HPLC method. The inter-day data were obtained by randomly choosing one replicate for each day.*

Day of analysis	Theoretical concentrations (µg mL^{-1})	Actual concentrations (µg mL^{-1}) (n=3)	SD	RSD%
Intra-day:				
Day 1	6.6	6.56	0.03	0.42
	13.2	13.15	0.04	0.31
	33.0	33.06	0.04	0.13
Day 2	6.6	6.55	0.04	0.53
	13.2	13.15	0.04	0.27
	33.0	33.05	0.04	0.12
Day 3	6.6	6.55	0.04	0.61
	13.2	13.13	0.05	0.38
	33.0	32.95	0.04	0.12
Inter-day:	6.6	6.55	0.04	0.61
	13.2	13.14	0.04	0.32
	33.0	33.02	0.02	0.06

Intermediate precision, which is the inter-day variation at the same concentration level, was determined on successive days. The intermediate precision for assay of (OXO-HCl) was found to be below 1.0% RSD (Table 2.). Robustness of a method is a measure of its capacity to remain unaffected by small variations in method conditions. The robustness of the proposed method was evaluated by altering pH of the mobile phase. The results (not shown) indicated lack of significant differences between the conditions of the method developed. The method is satisfactory for determination of (OXO-HCl) in syrup. These results also show that the filtration of the samples can be performed without loss of analyte.

Stability of standard solutions

Stability of standard and sample solutions was determined by monitoring the solutions of standard (OXO-HCl) and of a syrup solution sample over a period of two weeks [19]. The results showed that the retention times and signals were almost unchanged (RSD% <1.0) and that no significant degradation was observed within the given period, indicating the solutions were stable for at least two weeks.

Potentiometric method

In our Previous study [25], we have deduced that the ion-exchanger potassium tetrakis(*p*-chlorophenyl)borate has a highly exchange capacity, lipophilicity and ability to form counter-ions with many organic cations that differ in their stabilities. In this study Plasticized PVC membrane sensor incorporating T*p*-ClPB-oxomemazine ion- pair complex (Figure 5) was prepared with suitable solvent mediators and electrochemically evaluated as membrane sensor for oxomemazine under static mode of operations according to IUPAC recommendations [23]. The membrane was prepared using a casting solution of the composition 7.02: 63.16: 29.82 mass% of KT*p*-ClPB, *o*-nitrophenyl octyl ether or dioctyl phthalate and PVC, respectively. The two plasticizers have different dielectric constants. The sensor was soaked in drug solution and tested as oxomemazine sensor. Table 3 summarizes the potentiometric response characteristics of the sensor. It was found that the oxomemazine sensor plasticized with the two different plasticizers have almost the same characteristics. The sensor showed Nernstian response over the concentration range of 1.0×10^{-5} - 5.0×10^{-2} mol L^{-1} oxomemazine with cationic slopes of 62.5 and 61.6 mV $decade^{-1}$ for *o*-NPOE and DOP- based sensor, respectively. The detection limit was 1.0×10^{-5} mol L^{-1} with both plasticizers. Least squares analysis of the data gave the relationships:

$$E(mV) = 62.5 \log(oxo) - 261.6$$

and

$$E(mV) = 61.6 \log(oxo) - 293.3$$

for *o*-NPOE and DOP- based sensor, respectively. A typical calibration plot of the sensor was shown in Figure 6. The sensor displayed constant and stable potential readings within 0.2 mV from day to day and the calibration slope did not change by more than 1.0 mV $decade^{-1}$ over a period of 13 weeks. The sensor was useful for 12 and 13 weeks for *o*-NPOE and DOP- based sensor, respectively.

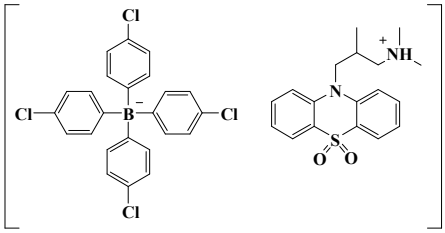

Figure 5. *Chemical structure of the ion- pair complex.*

Table 3. *Response characteristics of oxomemazine membrane sensor.*

Parameter	Value	
	***o*-NPOE**	**DOP**
Slope (mV $decade^{-1}$)	62.5	61.6
Intercept (mV)	261.6	293.3
Correlation coefficient (*r*)	0.9974	0.9993
Lower limit of detection(mol L^{-1})	1.0×10^{-5}	1.0×10^{-5}
Lower limit of linear range(mol L^{-1})	4.0×10^{-5}	6.0×10^{-5}
Working pH range	3.5 - 6.0	3.5 - 6.0
Life span/week	12	13

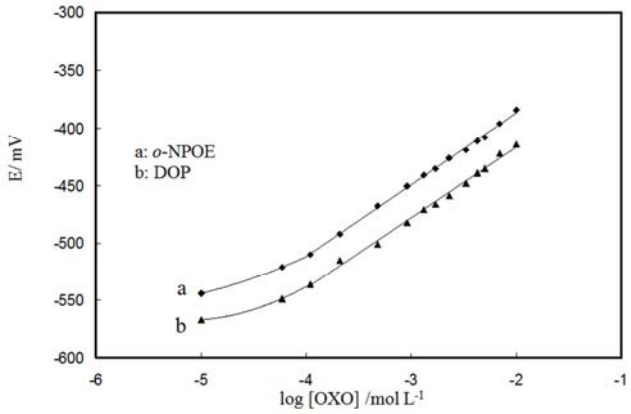

Figure 6. *Potentiometric response of oxomemazine membrane sensor.*

Effect of pH

The influence of pH on the potentiometric response of the sensor was studied using 10^{-4}, 10^{-3} and 10^{-2} M of oxomemazine solutions (Figure 7). The pH was adjusted by the addition of dilute hydrochloric acid or sodium hydroxide, as appropriate. From pH-potential profiles, it is evident that the potential readings are fairly constant over the pH range of 3.5 - 6.0. Within this acidic range, (OXO-HCl) is completely soluble, dissociated and sensed as a monovalent charged ion. At pH values lower than 3.5, the potential readings decreased due to interference by H^+ ions. At higher pH values (>6.0), progressive precipitation of the drug was observed.

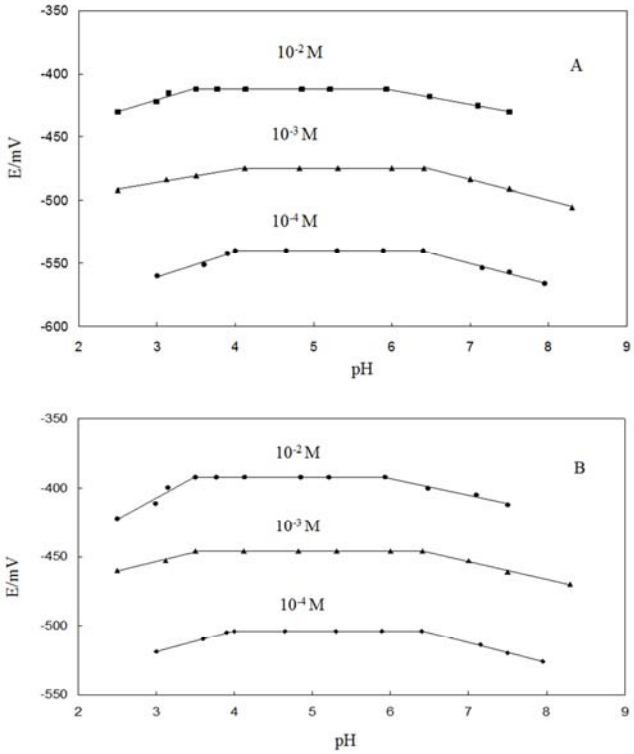

Figure 7. *pH – potential profile of oxomemazine membrane sensor. (A) DOP and (B) o-NPOE.*

Effect of foreign ions

The potentiometric response of oxomemazine sensor was tested in the presence of several inorganic cations.

Potentiometric selectivity coefficients $\left(k_{OXO,B}^{pot}\right)$ were used to evaluate the degree of interference. The data given in Table 4 were obtained using the separate solution method [23, 24] at 10^{-2} mol L^{-1} (OXO-HCl). It is clear that the sensor is highly selective for oxomemazine ions compared with some common cations. Pharmaceutical excipients and diluents (e.g., glucose, maltose, manitol, starch, talc powder and magnesium stearate) at concentration as high as 400-fold molar excess over oxomemazine did not interfere. The *o*-NPOE- based sensor was generally more selective than the DOP- based one.

Table 4. *Potentiometric selectivity coefficients* $\left(k_{OXO,B}^{pot}\right)$ *of oxomemazine membrane sensor.*

Interferent, B	$\left(k_{OXO,B}^{pot}\right)$	
	***o*-NPOE**	**DOP**
paracetamol	2.1×10^{-2}	1.4×10^{-2}
Glucose	2.6×10^{-3}	5.7×10^{-4}
Maltose	2.1×10^{-3}	4.4×10^{-4}
Urea	2.6×10^{-3}	2.1×10^{-2}
Ba^{+2}	2.8×10^{-3}	7.7×10^{-3}
Mg^{+2}	2.1×10^{-3}	6.9×10^{-3}
Na^{+}	2.6×10^{-3}	2.3×10^{-3}
NH_4^{+}	5.8×10^{-3}	6.2×10^{-4}
K^{+}	9.0×10^{-4}	1.8×10^{-4}
Ca^{+2}	1.2×10^{-3}	5.0×10^{-6}
Fe^{+2}	9.3×10^{-3}	8.7×10^{-3}

Determination of oxomemazine

The validity of the oxomemazine membrane sensor for the determination of oxomemazine was assessed by determining 3.7 µg - 3.7 mg mL^{-1} standard (OXO-HCl) solutions using the calibration graph method. The results obtained showed an average recovery of 100.65% and a mean standard deviation of 0.86% (n=5) using both solvent mediators –based membrane sensor. Oxomemazine (as antihistamine) in different pharmaceutical preparations was also determined (Table 5). Average recoveries of 100.4% and 100.9% of the nominal and mean standard deviation of 0.84% and 0.79% for the *o*-NPOE and DOP- based membrane sensor were obtained, respectively.

Table 5. *Determination of oxomemazine in pharmaceutical preparations by using oxomemazine membrane sensor.*

Trade name and source	Nominal content (g/100mL)	Recovery,%[a]	
		***o*-NPOE**	**DOP**
Toplexile "Amriya for Industries Alexandria-Egypt"	0.033	100.4 ± 0.2	100.9 ± 0.2

[a] Average of 5 measurements

4. Conclusion

From the present work we can conclude that there are different advantages where the sensor technique more simple and cheaper than HPLC techniques. On the other hand, HPLC gave a rapid (less than 6 min) analysis, high accuracy and precision, and lower LOD than sensor method and because of the good accuracy, no internal standard was needed. Both the two methods can be used for the determination of (OXO-HCl) in syrup without any interference.

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Conflicts of Interests

All authors have none to declare

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