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Short communication

A quantitative NMR protocol for the simultaneous analysis of atropine and obidoxime in parenteral injection devices

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ABSTRACT

A rapid selective and accurate quantitative ^{1}H NMR method was developed for the simultaneous analysis of obidoxime chloride and atropine sulfate, the active components in parenteral injection devices (PID) used for the emergency treatment of poisoning by toxic organophosphates. The spectra were acquired in 90% H_2O–10% D_2O using sodium 3-(trimethylsilyl)-1-propane sulfonate hydrate as the internal standard. Both synthetic mixtures and dosage forms were assayed. The results were compared with those obtained from a reported HPLC method.

1. Introduction

The overt or covert use of anticholinesterase compounds viz. nerve agents, in the battlefield poses a constant threat during wartime or terrorist attacks [1–4]. Hence, for the emergency treatment of poisoning by toxic organophosphates, auto injectors, which permit a rapid and convenient means for the intramuscular self-administration of atropine in combination with certain oximes viz. obidoxime or pralidoxime, have been introduced [5–10]. Defence Research and Development Establishment (Gwalior, India) has developed a reusable auto injector in which the cartridge can be replaced after the expiry of shelf life and the auto injector device can be reused. Several methods have been reported in the literature for the assay of active pharmaceutical ingredients of the drug cartridges [11–15]. Despite the availability of many LC-UV methods for the analysis of atropine and pyridinium oximes, we found that only a few of them can be applied directly for the simultaneous analysis of atropine and obidoxime chloride in drug cartridges [16–17]. Moreover, most of these methods rely on the development of an assay based on the LC-UV separation of the constituents of drug cartridges which require exotic and time consuming sample preparation, prior separation of the components, equilibration of the analytical platform and it suffers from memory effects. No pharmacopoeial method exists for the assay of obidoxime chloride and atropine sulfate in combination in drug cartridges. All of these drawbacks necessitated the development of a method which enables fast and simultaneous quantitative determination of active ingredients in drug cartridges. We thought of exploiting the virtues of quantitative NMR to overcome all the problems associated with the presently published methods. Quantitative NMR spectroscopy is a primary ratio method of measurement [18–19]. Several texts on the broad range of applications in pharmaceutical analysis have been published [20–22]. We, herein describe a method based on quantitative ^{1}H NMR that can be used for the simultaneous analysis of atropine and obidoxime in the drug cartridges. Applicability of the developed method was checked on drug cartridges (developed in DRDE, Gwalior) and results were compared to those obtained by the reported LC-UV method [17] validation parameters were derived and the results were compared with that for the NMR method.

2. Theory

The amount, W_X, of obidoxime chloride and atropine sulfate (in mg/ml) was calculated using the equation:

$$W_X = 1.59 \left(\frac{A_X}{A_{DSS}}\right)\left(\frac{N_{DSS}}{N_X}\right)\left(\frac{M_X}{M_{DSS}}\right) W_{DSS} \quad (1)$$

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W_X is the weight of analyte (per ml of solution), W_{DSS} is the weight of DSS (per 700 μl solution), A_X is the area of the integrals for the analyte, A_{DSS} is the area of the integrals for DSS, M_X is the molecular weights of the analyte and M_{DSS} is the molecular weights of DSS. The factor 1.59 is used for converting the units of W_X (from mg of analyte per 630 μl to mg of analyte per 1 ml of PID solution).

3. Experimental

3.1. Materials

Atropine sulfate (>99% pure), deuterated water deuteration degree min. 99.96% was purchased from Merck (Germany). Sodium 3-(trimethylsilyl)-1-propane sulfonate hydrate (DSS) (>99%), methyl-4-hydroxy benzoate (99%), ortho-phosphoric acid (99.999%) sodium dihydrogen phosphate (99%) and 1-octane sulfonic acid (∼98%) were obtained from Aldrich (USA). Obidoxime chloride was synthesised in-house and its purity (>99%) checked by LC-UV and spectroscopic techniques. Tetramethyl ammonium chloride (≥99%) was obtained from Fluka (Germany). Acetonitrile from J.T. Baker (Mexico) and distilled water, deionized by Milli-Q water purification system (Millipore, USA) were used for LC-UV mobile phase.

3.2. Instrumentation

NMR; Bruker av II operating at a frequency 400.13 MHz for protons, equipped with a 5 mm multinuclear inverse probehead and 5 mm multinuclear observe probehead.

LC-UV; Agilent 1100 liquid chromatograph equipped with: 7725 rheodyne injector with 20 μl loop, Hystar 3.1 software for data acquisition and processing, variable wavelength detector and a Zorbax Eclipse XDB-C_{18}, 125 mm X 4 mm with an average particle diameter of 5 μm was used.

3.3. Preparation of standard stock, test solutions for NMR and LC-UV analysis

A 300 mM solution of DSS was prepared immediately before use in 10 ml of D_2O. Stock solutions (5 ml) of obidoxime chloride (220 mg/ml) and atropine sulfate (30 mg/ml) were prepared in water. The solutions were diluted with water to obtain six different concentrations of the working solutions each for obidoxime (220.00, 110.00, 55.00, 27.50, 13.75, 6.87 mg/ml) and atropine sulfate (3.00, 1.50, 0.75, 0.37, 0.18, 0.09, 0.05 mg/ml). The NMR sample was prepared by taking 630 μl of the analyte solution 70 μl of the DSS solution in a NMR tube. For LC-UV analysis, stock solutions and their appropriate dilutions were prepared in mobile phase. All solutions were prepared immediately before analysis. The solutions were agitated on a vortex shaker before analysis.

3.4. LC-UV analysis

LC-UV analyses of the analytes were carried out in accordance to the method reported earlier [17].

3.5. NMR analysis

All the samples were locked and shimmed individually on 90% H_2O + 10% D_2O at a calibrated probe temperature of 20 °C so that the DSS signal achieved a linewidth no larger than 1.0–1.2 Hz. The relaxation time T_1 was determined for the protons of interest (Table 1, Fig. 1).

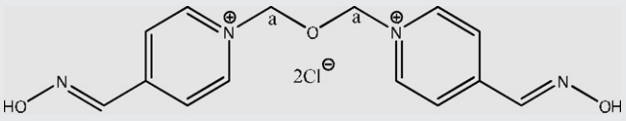
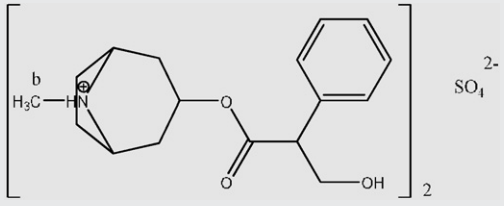
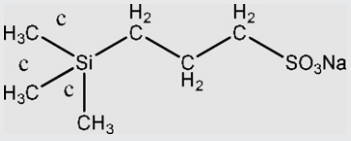
The *Ernst angle* α_e, for the pulse repetition time t_r (of 8 s) was optimized to for the T_1 of the longest relaxing DSS nuclei, to ensure maximum recovery of the transverse magnetization (Eq. (2)):

$$\cos\alpha_e = e^{-t_r/T_1} \quad (2)$$

The spectra were acquired in non-spinning mode, by zg0pr pulse program wherein suppression of the water signal was achieved. The carrier frequency was set on HOD and 4 dummy scans. 128 transients were recorded with 32k data points for each free induction decay (FID) and zerofilled to 64k data points. The FIDs were apodized with 0.2 Hz exponential line broadening function before fourier transformation. Manual two parameter phase correction was used to obtain high quality absorption line shape followed by baseline correction.

The active components were analyzed individually by ^{1}H NMR first to identify the peaks to be used for quantification (Table 1). These peaks of interest were integrated with respect to the internal standard for which an arbitrary constant value was attributed.

Table 1
Assignments of ^{1}H NMR resonances of obidoxime chloride, atropine sulfate and DSS used for quantification.

Component	Structure and peaks integrated for analysis	Number of protons involved in integration	Chemical shifts	Multiplicity	T_1
Obidoxime chloride		a = 4	6.37	Singlet	128.7 ms
Atropine sulfate		b = 3	2.75	Singlet	112.2 ms
DSS		c = 9	0.00	Singlet	2.6 s

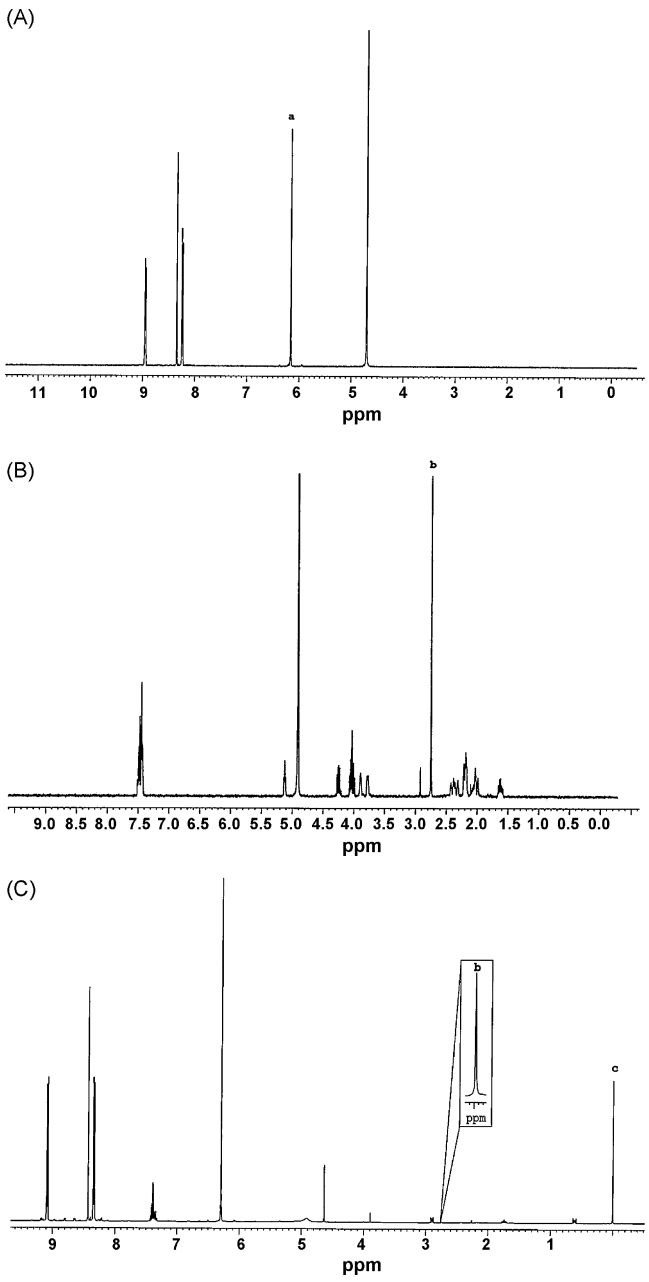


Fig. 1. ^{1}H NMR spectra of (A) obidoxime chloride (B) atropine sulfate and (C) drug cartridge formulation.

4. Results and discussion

The solutions were analyzed by the ^{1}H NMR and the reported LC-UV method. Then mixtures were prepared from the stock solutions and quantity of the analytes were measured (using Eq. (1)) taking different amounts of the internal standards. The results were compared with those obtained from the LC-UV method.

4.1. Linearity of calibration curves

Linearity of the ^{1}H NMR method was assayed by plotting fifteen calibration curves on fifteen different days analyzing seven standard solutions in the concentration range of 0.094–3 mg/ml ($r^2 \geq 0.99$) for atropine sulfate and 3.44–110 mg/ml ($r^2 \geq 0.99$) for obidoxime chloride. The reproducibility of the results was found to be excellent (results not shown). The same was done for the LC-UV method within the effective concentration range of 0.40–20 μg/ml and 0.022–3.3 μg/ml for atropine sulfate ($r^2 \geq 0.99$) and obidoxime chloride ($r^2 \geq 0.99$) respectively. As quantitative NMR is a primary ratio method, linearity is of little significance for assessing the specific detector response factor for different analytes. These experiments were mainly aimed at checking the system stability for the quantitative analysis on a large throughput basis during quality control procedures. Whereas, the calibration curves for LC-UV were used for quantification of the analytes.

4.2. Lower limit of quantification

Due to sufficiently high concentration in the drug cartridges, the limit of detection and quantification by ^{1}H NMR is not applicable in the strict sense, for the quality control purposes. The concentration of atropine sulfate (1 mg/ml) is much lower that that for obidoxime (110 mg/ml) in the drug cartridges. Hence, for optimization of quantitative ^{1}H NMR method for the simultaneous determination of the two analytes, lower limit of quantification (LLOQ) was estimated for atropine sulfate (21 μg/ml) at a signal to noise ratio of 10:1. The LLOQ of atropine sulfate was used as the threshold of dilution for the drug cartridges solution for further analysis. It is worth mentioning here that the signal line width and height of the signal are highly dependent on the spin–spin relaxation time, sample temperature and the magnetic field homogenity played a major role in determining the lower limits of quantitation of the analytes. These factors were effectively overcome by selecting a high flow rate of 535 l/min of the heating air and shimming the magnet so that the DSS signal achieved a linewidth <1.0 Hz. As for the LC-UV method, the upper and the lower limits of quantification for atropine sulfate were found to be 20.0 and 0.40 μg/ml whereas that for obidoxime chloride were found to be 3.3 and 0.022 μg/ml respectively.

4.3. Ruggedness

As reported elsewhere [23] there was no significant analyst influence on quantitative measurements. No quantitative differences were observed by positioning of the NMR tube in the turbine 4 mm above or 5 mm below the position recommended by the spectrometer manufacturer and incomplete and over filling of the sample tube by ±100 μl (making appropriate corrections for the volume change) were studied. Manual phase correction was performed carefully as it was found to have a major influence on the accuracy of the integrals. The analysis was also carried out on multinuclear broad band observe probehead to ascertain its effect. The only influence observed was that the LLOQ was marginally poor in this case.

4.4. Accuracy and precision

Accuracy was assessed by determining the different concentrations of the samples of atropine sulfate and obidoxime chloride relative to the known concentration of internal standard. Precision of the method were determined by measuring intraday and interday variations. The RSD values were found to be below 4% for atropine and 1% for obidoxime indicating good repeatability of the ^{1}H NMR (Table 2). Systematic errors were not observed.

4.5. Specificity and selectivity

Specificity of the ^{1}H NMR method was established first by quantifying one active component and then analyzing samples where the other components (including the excipients) were added one after the other, along with the previous ones, until finally the sample attained the desired composition of drug cartridges. For ascertaining specificity of the method, 2D NMR (HMBC and TOCSY) and

Table 2
Precision, accuracy[a] and recovery of atropine sulfate and obidoxime by NMR spectroscopy and HPLC from synthetic mixtures.

Sample number	DSS[b] (mg/ml)	Atropine sulfate (mg/ml)	Recovery (RSD %)				Obidoxime chloride (mg/ml)	Recovery (RSD %)			
			NMR		HPLC			NMR		HPLC	
		Taken	Interday ($n=3$)	Intraday ($n=3$)	Interday ($n=3$)	Intraday ($n=3$)	Taken	Interday ($n=3$)	Intraday ($n=3$)	Interday ($n=3$)	Intraday ($n=3$)
1	70.95	3.00	99.4 (2.0)	97 (2.1)			220	100.5 (0.5)	99.9 (0.4)		
2	35.47	3.00	101.2 (3.0)	98.6 (2.0)	99.8 (1.1)	99.5 (0.9)	220	98.9 (0.6)	99 (0.6)	99.5 (0.3)	99.3 (0.2)
3	17.74	3.00	102 (2.4)	100.2 (1.6)			220	99.1 (0.9)	99 (1.0)		
4	70.95	1.00	97.4 (2.2)	99 (1.9)			110	99.2 (0.8)	100 (0.3)		
5	35.47	1.00	98.9 (3.2)	99.4 (2.2)	99.9 (0.9)	99.6 (1.0)	110	101.4 (0.3)	102 (0.8)	99.6 (0.7)	99.8 (0.3)
6	17.74	1.00	99.0 (1.8)	101 (3.3)			110	100.0 (0.3)	99 (0.2)		
7	70.95	0.25	96.8 (3.2)	99 (3.4)			55	100.4 (1.0)	99 (0.9)		
8	35.47	0.25	101.1 (3.9)	99.0 (3.5)	99.6 (1.8)	99.7 (2.0)	55	102.0 (0.5)	99 (0.6)	99.4 (1.1)	99.6 (0.5)
9	17.74	0.25	99 (3.4)	101.8 (4.0)			55	101.5 (0.8)	102 (1.0)		

[a] Accuracy expressed as RSD %.
[b] Taken only for ^{1}H NMR analysis.

Table 3
Comparison of statistical results of the developed ^{1}H NMR method with HPLC method for drug cartridges.

Vial number	Atropine labeled strength (mg/ml)	NMR method	HPLC method	Obidoxime chloride labeled strength (mg/ml)	NMR method	HPLC method
		Mean (RSD%) ($n=5$)	Mean (RSD%) ($n=5$)		Mean (RSD%) ($n=5$)	Mean (RSD%) ($n=5$)
1.	1.00	1.01 (1.92)	1.02 (1.52)	110.00	110.20 (0.96)	110.24 (0.90)
2.	1.00	0.98 (1.75)	0.98 (1.31)	110.00	109.50 (1.01)	109.85 (0.89)
3.	1.00	0.99 (1.89)	1.00 (1.35)	110.00	109.92 (1.14)	110.01 (0.96)
4.	1.00	1.01 (1.86)	0.99 (1.01)	110.00	108.95 (1.10)	109.10 (0.87)
5.	1.00	0.94 (1.84)	0.97 (1.40)	110.00	110.02 (1.05)	110.29 (0.79)
6.	1.00	0.99 (1.96)	0.99 (1.32)	110.00	110.68 (1.19)	110.86 (1.01)
7.	1.00	1.03 (1.73)	1.02 (1.42)	110.00	109.83 (1.29)	110.01 (0.97)
8.	1.00	1.00 (1.94)	1.02 (1.51)	110.00	109.88 (0.96)	109.99 (0.82)
9.	1.00	0.99 (1.79)	1.00 (1.33)	110.00	109.75 (1.05)	109.89 (0.89)
10.	1.00	0.97 (1.40)	0.98 (1.00)	110.00	110.10 (1.03)	109.98 (0.92)

^{1}H NMR experiments consequent to spiking of the standard compounds in the drug formulation, supported the assignment of peaks used for quantification of the mixture. Representative ^{1}H NMR spectra clearly demonstrate the specificity and selectivity of the method (Fig. 1). The method was found to be selective as spectral overlap of the analytes of interest was neither found with themselves nor with the excipient. These tests were carried out for all the samples that are depicted in Table 2. LC-UV method [17] as well produced well resolved peaks for all the analytes.

4.6. Recovery

Recovery experiments were conducted within the quantification limit of the analytes to determine the accuracy of the method for quantification of obidoxime and atropine (Table 2). The quantity of the internal standard and the analytes were varied and interday, intraday recoveries were calculated to observe the influence of the quantity of the internal standard on the results. The relative proportions of the internal standard and the analyte did not have any effect on accuracy of the method. The results indicate that the average intraday and interday recoveries were found to be 99.5 and 99.3 for atropine sulfate and 100.3 and 99.9 for obodoxime chloride for the ^{1}H NMR analysis, whereas, the recoveries for the LC-UV method, average intraday and interday recoveries of atropine sulfate and obodoxime chloride were found to be 99.8, 99.6% and 99.5, 99.6% respectively.

5. Application of the method

One dose (2.1 ml) of drug cartridge, developed in our laboratory, had the following composition: 1 mg/ml atropine sulfate and 110 mg/ml of obidoxime chloride as active ingredients along with 0.1% (w/v) of methyl-4-hydroxy benzoate in pyrogen free water. Finally, efficacy of the quantitative ^{1}H NMR method was compared with that obtained from the reported LC-UV method [17]. Ten drug cartridges (developed by DRDE) were randomly selected from different batches. The results were found to compare well (Table 3). The representative ^{1}H NMR spectra of the drug cartridges solution is shown in Fig. 1(C).

6. Conclusion

Atropine sulfate and obidoxime chloride in drug cartridges can be determined by ^{1}H NMR with the use of DSS as internal standard. The method is simple, selective, rapid and gives a clear picture about all the components present in the formulation in a single experiment as compared to the reported LC-UV and LC-UV-GLC methods of analysis.

References

[1] M. Nagao, T. Takatori, Y. Matsuda, M. Nakajima, H. Iwase, K. Iwadate, Toxicol. Appl. Pharmacol. 144 (1997) 198–203.
[2] T. Okumura, T. Hisaoka, A. Yamada, T. Naito, H. Isonuma, S. Okumura, K. Miura, M. Sakurada, H. Maekawa, S. Ishimatsu, N. Takasu, K. Suzuki, Toxicol. Appl. Pharmacol. 207 (2005) 471–476.
[3] C. Macilwain, Nature 363 (1993) 3.
[4] A.T. Tu, J. Mass Spectrom. Soc. Japan 44 (1996) 293–320.
[5] V. Riihimaki, E. Kantolahti, R. Vaisakorpi, in: K. Koskenvuo (Ed.), Kenttalaakinta. Ensihoidon Perusteet, Finnsh Defense Forces, Hameenlinna, 1993, pp. 494–500.
[6] R. Vijayaraghavan, N. Jain, A. Gautam, M. Sharma, S. Singh, D. Kumar, R. Singh, P. Kumar, A.S.B. Bhaskar, A.K. Gupta, S. Jain, J. Med. CBR Def. 5 (2007) 1–12.
[7] P. Kumar, R. Vijayaraghavan, D. Kumar, N. Jain, H.M. Swarnkar, C.K. Waghmare, B.K. Bhattacharya, M. Sharma, S. Jain, Curr. Trends Biotechnol. Pharm. 2 (2008) 251–259.
[8] R.E. Gosslin, H.C. Hodge, R.P. Smith, M.N. Gleason, Clinical Toxicology of Commercial Products, 4th ed., Williams and Wilkins, Baltomore, 1976.
[9] F. Hobbiger, in: G.B. Koelle (Ed.), Handbuch der Experimentellen Pharmakologie (Cholinesterases and Anticholinesterases), vol. 15, Springer, Heidelberg, 1963, pp. 921–988.
[10] G. Puu, E. Arturssun, G. Bucht, Biochem. Pharmacol. 35 (1985) 1505–1510.

[11] C. Grasshoff, H. Thiermann, T. Gilssen, T. Zilker, L. Szinicz, J. Chromatogr. B 756 (2001) 203–208.
[12] J. Pahjola, M. Harpf, J. Chromatogr. A 686 (1994) 350–354.
[13] U. Spohrer, P. Eyer, J. Chromatogr. A 693 (1995) 55–61.
[14] H.P. Benschop, K.A.G. Konings, S.P. Kosses, D.A. Ligtense, J. Chromatogr. 225 (1981) 107–114.
[15] C.L. Briggs, K.J. Simons, J. Chromatogr. 257 (1983) 132–136.
[16] N.D. Brown, L.L. Hall, H.K. Sleeman, B.P. Doctor, G.E. Demaree, J. Chromatogr. 148 (1978) 453–457.
[17] B.M. Paddle, M.H. Dowling, J. Chromatogr. 648 (1993) 373–380.
[18] T.J. Quinn, Metrologia 34 (1997) 61–65.
[19] F. Malz, H. Jancke, J. Pharm. Biomed. Anal. 38 (2005) 813–823.
[20] U. Holzgrabe, R. Deubner, C. Schollmayer, B. Waibel, J. Pharm. Biomed. Anal. 38 (2005) 806–812.
[21] W.K. Bernd, F. Diehl, U. Malz, Holzgrabe, Spectrosc. Asia 3 4 (2007) 15–19.
[22] U. Holzgrabe, I. Wawer, B. Diehl, NMR Spectroscopy in Drug Development and Analysis, Wiley-VCH Verlag GmbH, Weinheim, 1999.
[23] G. Maniara, K. Rajamoorthy, S. Rajan, G.W. Stockton, Anal. Chem. 70 (1998) 4921–4928.