http://www.e-journals.in
DOI:10.7598/cst2015.4624

Chemical Science Transactions
2015, **4(1),** 270-274

RESEARCH ARTICLE

Assay of Dexmedetomidine in Bulk Samples and Pharmaceutical Formulations by Extractive Spectrophotometry

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Received 3 September 2014 / Accepted 17 October 2014

Abstract: Three simple and sensitive spectrophotometric methods (A-C) for the assay of dexmedetomidine in pure and dosage forms based on the formation of chloroform soluble ion-associates under specified experimental conditions are described. Three acidic dyes, namely Wool Fast Blue BL (WFB BL, method A), Tropaeolin ooo (Tpooo, method B), Naphthol Blue 12BR (NB 12BR, method C) were utilized. The extracts of the ion-associates exhibit absorption maxima at 580 nm, 480 nm, and 590 nm for methods A, B and C respectively. Beer's law and the precision and accuracy of the methods are checked by the UV reference method. The results are reproducible with an accuracy of ±1.0%. The methods are found to be suitable for the determination of dexmedetomidine in the presence of the other ingredients that are usually present in dosage forms.

Keywords: Dexmedetomidine, Ion-associates, solubility, Spectrophotometry

Introduction

Dexmedetomidine[1] hydrochloride (DEX) is the S-enantionmer of medetomidine which is a α_2-adrenergic agonist and is chemically described as (+)-4-(*S*)-[1-(2,3-dimethylphenyl)-ethyl]-1*H*-imidazole monohydrochloride. Literature mentions a few methods such as high performance liquid chromatography[2,3], and LC-MS[4-6] for its determination in biological fluids and dosage forms. Although spectrophotometric methods are the instrumental methods of choice commonly used in industrial laboratories, no colorimetric method has been reported so far for the determination of dexmedetomidine. Therefore, the need for a

fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing dexmedetomidine. The present paper describes three simple and sensitive extraction spectrophotometric methods for the determination of dexmedetomidine, based on its tendency to form chloroform extractable ion-association complexes with acidic dyes WFB BL, Tpooo and NB 12BR under specified experimental conditions by exploiting the basic nature of the drug molecule.

Experimental

Instruments

A Systronics 166 digital UV spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements. An Systronics 361 digital pH meter was used for pH measurements.

All reagents and chemicals used were of analytical grade and doubly distilled water was used throughout. Aqueous solutions of WFB BL (BDH, Mumbai, India, 0.2%) TPooo (BDH, Mumbai, India, 0.2%) and NB 12BR (BDH, Mumbai, India, 0.2%) were prepared by dissolving the required amount in doubly distilled water. The solutions were washed with chloroform to remove the chloroform-soluble impurities and the residual solvent was removed by bubbling with nitrogen.

Buffer solutions

The glycine-HCl buffer[7] solutions (pH 1.3 for method C and pH 1.5 for method A were prepared.

Preparation of standard drug solution

A one mg/mL solution was prepared by dissolving 100 mg of pure DEX in 100 mL of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations 100 μg/mL for M_1, M_2 and M_3 respectively.

Recommended procedures

In to a series of 125 mL separating funnels containing aliquots of standard DEX solution [0.5-2.5 mL, 100 μg/mL (M_1, M_2 or M_3),] 6.0 mL of buffer solution pH 1.5 (M_1, or M_3) or 0.1M HCl (M_2) 2.0 mL of dye solution [WFB BL (M_1); TPooo (M_2); NBB (M_3)] were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0 mL with distilled water and 10 mL of chloroform was added. The contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 590 nm (M_1), 480 nm (M_2), 620 nm (M_3) against the reagent blank. The amount of DEX was calculated from the calibration plot

Results and Discussion

The optimum conditions for the color development in each method were established by varying the parameters one at a time[8], keeping the others fixed and observing the effect produced on the absorbance of the colored species[9].

Optimum conditions fixation

Conditions under which the reaction of DEX with each dye fulfills the essential analytical requirements were investigated. All the experimental conditions studied were optimized at room temperature ($25 \pm 3\ ^0$C) and were established by varying one parameter at a time (10) and observing its effect on the absorbance of the colored species.

Different organic solvents such as benzene, toluene, nitrobenzene, carbon tetrachloride, 1,2-dichloromethane, chloroform, ethyl acetate and isobutyl ketone were tested for the extraction of the ion-association complex formed between the DEX and each dye. Chloroform was suggested as the solvent of choice for the extraction of the colored complex with respect to maximum stability.

In order to establish the optimum pH range (for M_1-M_3), the DEX was allowed to react with the respective dye in aqueous solution buffered between pH 1.0-10.0 and the complex formed was extracted into chloroform for absorbance measurement. The results show that a quantitative extraction was produced between pH 1.1-1.5 (for M_1, M_3) or 0.1M HCl (for M_2). All subsequent studies were carried out at pH 1.5 (for M_1, M_3) or 0.1M HCl (for M_2). The volume of this buffer added (4-10 mL) had no effect in methods for M_1, M_2, (pH 1.5) and M_3 (0.1M HCl) respectively. A 6.0 mL portion of buffer was found to be optimal in methods M_1, M_2, and M_3. The minimum shaking time was determined by varying the shaking time from 1-10 min; although 1 min was sufficient, prolonged shaking had no adverse effect on the extraction and 2 min was selected for this study. A ratio of 2:3 of organic to aqueous phases was required for efficient extraction of the colored species and lower reagent blank reading. It was found that better reproducibility and lower reagent blank were achieved if the dye was purified by extraction with chloroform initially. The color products were stable up to 30 min. The stoichiometric ratio of the DEX to dye was found as 1: 1 with WFB BL or TPooo and 2:1 with NB 12BR through slope analysis method.

Chemistry of the colored species

DEX possesses tertiary nitrogen, involves in ion association complex formation with an acid dye (WFB BL, M_1; Tpooo, M_2; NB 12BR, M_3), which is extractable into chloroform. The quantitative measure of the effect of complexation on acid-base equilibrium is most likely to be interpretable in terms of electronic, steric and other effects of complexing. The possible structure of the ion-association complex in each instance was established based on the analogy reports for similar types of molecules with acidic dyes and was further confirmed by slope-ration studies. The protonated nitrogen (positive charge) of the drug molecule in acid medium is expected to attract the oppositely charged part (negative charge) of the dye and behave as a single unit being held together by electrostatic attraction. Based on analogy the structure of ion association complexes are shown in Figure 1.

Interference studies

The interference studies in the determination of DEX in pharmaceutical formulation revealed that the normally existing excipients and additives like starch, lactose, gelatin, talc, magnesium stearate, aluminum hydroxide, sorbitol, calcium silicate and glycerin do not interfere even when present in excess than the anticipated amount. However, a preliminary clean up procedure with chloroform is necessary to avoid interference due to the presence of reducing sugars like lactose if present, prior to the estimation of DEX in formulations for method A, B and C respectively.

Analytical data

The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity for the method are given in Table 1. The precision of the method was found by measuring absorbances of six replicate samples containing known amounts of drug. Regression analysis using the method of least squares was made to evaluate the parameters. The accuracy of the methods was ascertained by comparing the results by the reference

method (Table 2). This comparison shows that there is no significant difference between the results of studied methods and those of the reference one.

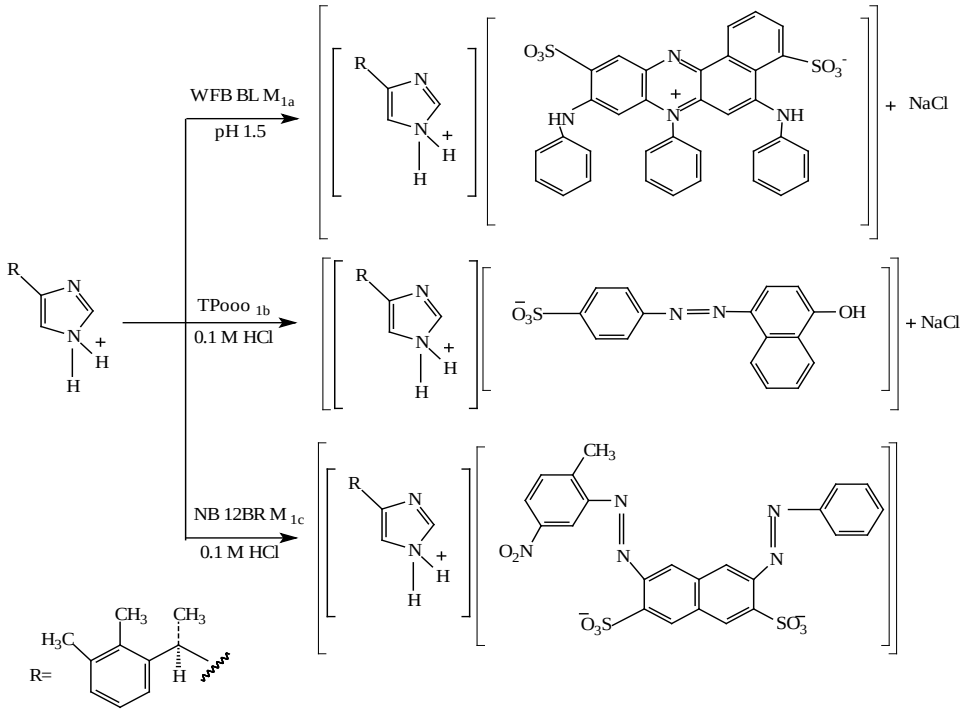

Figure 1. Chemistry of coloured species for DEX with dyes

Table 1. Optical Characteristics, Precision and Accuracy of the Proposed Methods for Dexmedetomidine

Parameters	Method M_1 WFB BL	Method M_2 TPooo	Method M_3 NB 12 BR
λ_{max} (nm)	590	480	620
Beer's Law limits (μg/mL)	1 – 10	1-6	1-8
Molar absorptivity (l $mol^{-1}cm^{-1}$)	1.63×10^4	2.98×10^4	1.95×10^4
Sandell's sensitivity (μg/cm^2/0.001 absorbance unit)	0.014	0.008	0.012
Regression Equation (y = a + bc)			
Slope (b)	0.0687	0.1261	0.0826
Standard Deviation on slope (S_b)	7.5×10^{-4}	5.5×10^{-4}	7.1×10^{-4}
Intercept (a)	0.0022	0.0001	0.0004
Standard Deviation on intercept (S_a)	3.2×10^{-3}	1.8×10^{-3}	3.01×10^{-3}
Standard Error of Estimation (S_e)	1.89×10^{-3}	1.7×10^{-3}	2.2×10^{-3}
Correlation coefficient (r)	0.9998	0.9999	0.9998
Relative Standard Deviation*	0.361	0.211	0.341
% error in bulk sample (95% confidence limit)**	-0.036	0.124	-0.121

**Average of six determinations considered.* ***Average of three determinations.*

Table 2. Assay of dexmedetomidine in pharmaceutical formulations

Pharmaceutical sample (Labeled amount)	% Recovery (mg)			
	Proposed method			Ref. Method
	M_1	M_2	M_3	
Inj_1 (100 mcg)	99.97 ± 0.49 t = 1.1 F = 1.27	99.89 ± 0.37 t = 1.54 F = 1.35	99.20 ± 0.88 t = 0.62 F = 1.58	99.51 ± 0.70
Inj_2 (100 mcg)	99.63 ± 0.21 t = 1.71 F = 2.67	99.81 ± 0.17 t = 0.98 F = 2.01	99.28 ± 0.54 t = 1.31 F = 2.03	98.93 ± 0.77
Inj_3 (100 mcg)	100.13± 0.63 t = 1.16 F = 2.06	99.88 ± 0.54 t = 0.46 F = 1.54	99.25 ± 0.65 t = 0.75 F = 1.83	99.48 ± 0.88
Inj_4 (100 mcg)	99.27 ± 0.21 t = 0.22 F = 2.57	99.86 ± 0.24 t = 0.99 F = 3.72	99.56 ± 0.69 t = 0.15 F = 1.88	98.9 ± 0.35

Average (± RSD) of six determinations; the t and F values refer to comparison of the proposed method with the reference method; theoretical values at 95% confidence limits, t = 2.57, F = 5.05.

Conclusion

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi component mixture. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibilities in the assay of a particular component in a complex dosage formulation. In the present study, DEX was determined successfully as a pure compound as well as a component in representative dosage formulation. The proposed methods are simple, selective and can be used in the routine determination of DEX in bulk samples and formulations with reasonable precision and accuracy.

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