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Sensitive Spectophotometric Methods for the Analysis of Some Anesthetic Drugs

N. D. DINESH, P. NAGARAJA AND K. S. RANGAPPA*
Department of Studies in Chemistry,
University of Mysore, Manasagangotri, Mysore-570 006.

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Two simple and sensitive visible spectrophotometric methods for the analysis of some anesthetic drugs in pure and available pharmaceutical preparations have been developed. The first method is based on the formation of colored ion-pair complexes by the drugs, ketamine hydrochloride, lignocaine hydrochloride, bupivacaine hydrochloride and tetracaine hydrochloride with bromothymol blue. The ion-pair complexes formed are quantitatively extracted into dichloromethane and absorbance is measured at 420 nm. The second method is based on the coupling of the diazotized drugs benzocaine and procaine hydrochloride with a new and highly sensitive coupling agent, monosodium salt of 4-amino-5-hydroxynaphthalene-2,7-disulfonic acid. The absorbance of the red azo-dye is measured at 530 nm. These methods are quantitatively evaluated and found to be precise and accurate. Beer's law is obeyed in the concentration range 1-15 μg/ml and 0.1-7 μg/ml for first and second methods, respectively.

Anesthetics are drugs, which produce anesthesia, a condition of inability to feel sensation[1]. Two types of anesthetic drugs are generally recognized: local and general. Local anesthetic drugs can be conveniently divided into two groups: esters and nonesters, benzocaine (BzC), tetracaine hydrochloride (TC) and procaine hydrochloride (PC) belong to p-aminobenzoic acid ester group, whereas lignocaine hydrochloride (LC) and bupivacaine hydrochloride (BpC) are nonester or amide type anesthetic drugs. Local anesthetics with an ester linkage and those with amide linkage differ significantly in hypersensitivity, metabolism and duration of action[1]. Ketamine hydrochloride (KT) is a general anesthetic drug.

*For correspondence
E-mail: rangappaks@yahoo.com

The official method of IP describes nitrite titration method for BzC[2], BpC[3], KT[4], LC[5] and PC[6] (TC is not included in IP) and USP describes HPLC method for BzC[7], TC[7], BpC[8], KT[9], LC[10] and PC[11]. A few contemporary analytical techniques employed for the analysis of these drugs include HPLC[12], chemiluminescence[13], potentiometry[14] and titrimetry[15,16]. A few spectrophotometric methods have already been reported for the determination of anesthetic drugs. Reported methods in one way or the other have disadvantages like lack of sensitivity[17-19], long time for reaction to complete[20] and tedious heating procedures[21]. However, no single method has

been reported for the analysis of all these local anesthetic drugs. It is interesting to note that, no spectrophotometric method has been reported for the assay of KT. The present methods are attempts to overcome the shortcomings of the existing methods and succeeded in developing rapid and highly sensitive spectrophotometric methods for the analysis of some anesthetic drugs in pure and dosage forms.

A JASCO model UVIDEC-160 UV-VIS spectrophotometer with 1 cm matched cells were used for electronic spectral measurements. LC and BpC were obtained as gift samples from Astra-IDL, Bangalore and KT, TC, BzC and PC used were procured from Sigma Chemical Co, St. Louis, MO. 4-Amino-5-hydroxynaphthalene-2,7-disulfonic acid (ANSA) used was obtained from E. Merck, Mumbai and all other reagents used were of AR grade. Deionized water was used to prepare all solutions. Standard solutions of drugs (1000 μg/ml) were prepared by dissolving 100 mg each of drug in distilled water and diluting to mark in 100 ml calibrated flask. A working standard solution of each drug containing 25 μg/ml was prepared by further dilution. Solutions of the samples were prepared by diluting the accurately measured volume of the samples (injections) with deionized water to get 1000 μg/ml of the drug solution. Suitable aliquots of these solutions were further diluted to get 25 μg/ml of the drug solution. For extraction method, 0.02% of bromothymol blue (first dissolved with 2-3 drops of ethanol and then diluted with water) and pthalate-sodium hydroxide buffer of pH 4.6 were prepared. For diazotization method, 0.1% ANSA, 0.1% sulfamic acid, 0.01% sodium nitrite, 4 M hydrochloric acid and 1 M sodium hydroxide were prepared in water. Freshly prepared solutions were always employed.

For extraction, 3.5 ml of bromothymol blue and 3 ml of pthalate-sodium hydroxide buffer of pH 4.6 were added to various amounts (10 to 150 μg) of each drug solutions (working standard) taken in a series of separating flasks, followed by the addition of 3 ml of dichloromethane. The contents were shaken well for 1 min and kept aside for another min. After that the separated organic phases were transferred to 50 ml beakers, aqueous phases were again extracted twice with 3 ml of the same solvent. The successive extracts were mixed well, dried over anhydrous sodium sulfate and transferred to 10 ml volumetric flasks. Then the solutions were made up to the mark with the same solvent and mixed well. The absorbance of these solutions were measured at 420 nm against a blank solution in the same manner and then calibration graphs were constructed.

For diazotization method, 1 ml of 4 M hydrochloric acid

TABLE 1: THE OPTICAL PARAMETERS AND PRECISION DATA.

Parameters	Extraction method				Diazotization method	
Drugs analyzed	KT	LC	BpC	TC	BzC	PC
Color	Yellow	Yellow	Yellow	Yellow	Red	Red
λmax (nm)	420	420	420	420	530	530
Stability	12 h	12 h	12 h	12 h	1 week	1 week
Beer's law range (μg/ml)	1-15	1-15	1-15	1-15	0.25-6	0.25-7
Molar absorptivity (L/mol/cm)	$1.3x10^4$	$1.1x10^4$	$1.2x10^4$	$1.1x10^4$	$2.0x10^4$	$2.5x10^4$
Sandell's sensitivity (μg/cm^2)	0.0227	0.0257	0.0274	0.0284	0.0082	0.0096
Detection limit (μg/ml)	0.25	0.25	0.5	0.5	0.05	0.1
Regression equation (y*)						
Slope (a)	0.1001	0.1010	0.0964	0.0938	0.1077	0.1001
Intercept (b)	0.0084	0.0062	0.0080	0.0104	0.0136	0.0067
Correlation coefficient(r)	0.9991	0.9995	0.9991	0.9990	0.9991	0.9992
R.S.D. (%)**	1.02	1.08	1.14	1.12	1.02	0.90

*y=ax+b, where y is the absorbance, a is the slope, b is the intercept and x is the concentration of drugs in μg/ml. **Expressed as average of five replicate determinations.

and 1 ml of 0.01 % sodium nitrite were added to a series of 25 ml standard flasks, kept in ice bath, containing 1.25-175 μg aliquot solution of the drug and mixed thoroughly. After 3 min, 1.5 ml of 0.1 % sulfamic acid was added, shaken well and flasks were removed from the ice bath. Then 1 ml of 0.1% ANSA and 2 ml of 1 M sodium hydroxide were added and the flasks were swirled well. Flasks were allowed to stand for further 1 min for maximum color development and then diluted up to the mark with water. After mixing the solutions thoroughly, absorbance was measured at 530 nm against a blank and calibration graphs were constructed. For both the methods, a suitable volume of the sample solutions containing 25 μg/ml of the drug was taken and the recommended procedure was followed for the analysis of drug.

The optical parameters and precision data for all the six drugs studied are given in Table 1. It was found that, for extraction method, 3 ml of 0.02% solution of bromothymol blue and 3 ml of pthalate-sodium hydroxide buffer of pH 4.6 were necessary to achieve the maximum color intensity of the product. Carbontetrachloride, chloroform, dichloromethane were tested as extractive solvents for the proposed reaction. Dichloromethane was preferred to other for its selective extraction. It offers advantages such as being economically cheaper and convenient to be used as extractive solvent.

For diazotization method, use of the 4 M hydrochloric acid was found to give better results than sulfuric acid for diazotization and its optimum volume was found to be 1-1.5 ml, higher concentration of which was found to decrease the intensity of the color. Therefore, 1 ml of 4 M hydrochloric acid was used for all measurements. A volume of 1 ml of 0.01 % sodium nitrite was found to be sufficient for complete diazotization, higher concentration of which did not affect the intensity of the color since excess was destroyed by adding 1.5 ml of 0.1% sulfamic acid. However, excess of sulfamic acid was found to have no effect on the color intensity. The optimum concentration of ANSA leading to a maximum intensity and stability of the color was found to be 1.0 ml of 0.1 %, higher concentration of which did not affect the stability and intensity of the color. 1.5-2.5 ml of 1.0 M sodium hydroxide was found to be necessary to give stable and intense red color. Therefore, 2.0 ml was used for all measurements. Color intensity was found to decrease below the lower limit and above the upper limit.

The effect of the concentration of excipients and additives associated with the drugs in its formulations were investigated by taking LC and BzC as model compounds for respective methods. These methods does not suffer any interference from commonly associated excipients and additives such as sucrose, lactose, dextrose, starch, magnesium stearate, sodium alginate and ascorbic acid (Table 2).

The applicability of the method for the assay of avail-

TABLE 2: DETERMINATION OF DRUGS IN PRESENCE OF EXCIPIENTS.

Method	Extraction method (LC added 7 μg/ml)*		Diazotization method (BzC added 3 μg/ml)*	
Excipients	Amount in mg/ml	% Recovery of LC ± % RSD**	Amount in mg/ml	% Recovery of BzC ± RSD**
Dextrose	35	101.6 ± 1.10	50	100.6 ± 1.10
Lactose	45	99.2 ± 1.41	40	99.8 ± 0.92
Sucrose	40	100.2 ± 1.46	45	101.0 ± 1.10
Starch	25	98.8 ± 0.76	30	99.4 ± 1.42
Magnesium stearate	40	99.6 ± 0.84	45	101.2 ± 0.84
Sodium alginate	25	99.8 ± 0.90	30	100.2 ± 0.96
Ascorbic acid	30	102.0 ± 1.60	40	103.4 ± 1.10

*For extraction method, 7 μg/ml of LC was added to a series of separating flasks containing different concentration of excipient and for diazotization method, 3 μg/ml of BzC was added to a series of 25 ml standard flasks containing different concentration of excipient and recommended procedures were followed for the analysis of drugs. Excipients up to concentration given in columns 2 and 4 respectively for extraction and diazotisation methods were not interfere in the analysis. **Expressed as average of five replicate determinations.

TABLE 3: ANALYSIS OF AVAILABLE COMMERCIAL FORMULATIONS.

Commercial formulations analyzed	Drug content	Label claim (mg/ml)	Amount of drug found in mg* (expressed as percent)	
			Proposed method	Official method
Extraction method				
Injections				
Xylocaine[a]	LC	21.3	99.90 ± 0.30	99.71 ± 0.50
Sensorcaine[b]	BpC	5.0	101.00 ± 0.30	97.00 ± 0.09
Ketamax[c]	KT	50	100.40 ± 0.20	98.20 ± 0.12
Ketalar[d]	KT	10	100.50 ± 0.10	99.00 ± 0.20
Diazotization method				
Injection				
Anesthone[e]	BzC	10.0	99.00 ± 0.28	98.50 ± 0.50

*Expressed as average of five replicate determinations ± RSD. Commercial formulations are marketed by, Astra-IDL (a and b), Troikaa Parentarel Pvt. ltd. (c), Parke-davis (d) and Neon labs (e).

able pharmaceutical formulations was examined. The results of the assay of some available formulations are summarized in Table 3. The results clearly indicate the utility of the proposed methods for the analysis of these drugs in pure and dosage forms.

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