SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF BUPIVACAINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS

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Bupivacaine hydrochloride is an anesthetic with prolonged duration of action. The aim of this study was to develop a fast, simple and sensitive spectrophotometric method for the determination of bupivacaine in pharmaceutical preparation. The maxim absorbance was finding at 262 nm. At the same time, a validation study was conducted for the proposed method, in accordance with the bioanalytical method validation guidelines. The studied validation characteristics showed that the proposed method can be successfully applied to pharmaceutical preparations analysis.

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Introduction

Bupivacaine (Figure 1), (*RS*)-1-butyl-*N*-(2,6-dimethylphenyl) piperidine-2-carboxamide, is an amide anesthetic with prolonged duration of action. It works by blocking the transmission of nerve impulses from sensory nerve endings and at high doses in motor nerve endings.

Bupivacaine HCl is used in sterile isotonic solution for local infiltration, peripheral nerve block and epidural and caudal to. Bupivacaine is indicated for the induction of local or regional anesthesia or analgesia in the surgery, oral surgery procedures, diagnostic and therapeutic procedures in obstetric interventions. Its action installs slower (5-10 min.) and anesthesia maintained longer compared to other anesthetics (3-8 hours depending on dose and site of administration). Mechanism of action is determined by the stabilization of neuronal membranes, which prevents appearance and conduct nerve impulses. Initially successful inhibit thermal sensitivity, tactile and proprioceptive and then - and neuro-muscular conduction. Analgesic action is prolonged after cessation of anesthesia, which decreases the need for postoperative analgesia in.

This should be done with caution in patients with hepatic impairment (is metabolized in the liver) in patients with renal and/or heart disease, epilepsy, shock, hypovolemia, myasthenia gravis, old. Dosage varies depending on the type of anesthesia, clinical history and physical condition of the patient, taking into account the risk of reaching toxic plasma concentration or cause damage to local nerves.[1-5]

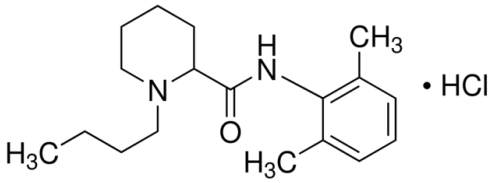

Figure 1. Chemical structure of bupivacaine hydrochloride

The literature describes other methods for the determination of bupivacaine hydrochloride like HPLC, MS.[6-9] These methods are modern, speed, but involves complex instruments which could not be available in most of laboratories. The aim of this study was to develop a simple, fast, cheap and sensitive spectrophotometric method for the determination of bupivacaine in pharmaceutical preparation.[10,11] In order to validate the proposed method, we investigated the following validation parameters: linearity, limit of detection, limit of quantification, precision and accuracy in accordance with the bioanalytical method validation guidelines.[12,13]

Experimental

Chemicals and reagents

Bupivacaine hydrochloride was supplied by Sigma Aldrich (USA), double distilled water, pharmaceutical preparation was provided from the pharmacy (ampoules of 5 mg mL^{-1}, 5 ml/ampoule).

Instrumentation

A Jasco V 530 double beam UV-Vis spectrophotometer was used. All the measurements was made in 1.0 cm quartz

Table 1. Absorbance values for linearity study

mg mL^{-1}	0.1	0.3	0.4	0.5	0.6	0.7	0.8	1
Absorbance I	0.1158	0.3344	0.4454	0.5592	0.6639	0.7809	0.888	1.0955
Absorbance II	0.1154	0.3337	0.4452	0.5594	0.6641	0.7803	0.8872	1.0959
Absorbance III	0.1157	0.3324	0.4456	0.5589	0.6631	0.7807	0.8881	1.0954
Average	0.1156	0.3335	0.4454	0.5591	0.6637	0.7806	0.8877	1.0956

cells at a scan speed of 1000 nm min^{-1} and scan range of 200 – 400 nm, fixed slit width of 2 nm. Scanning of standard solutions of bupivacaine hydrochloride (mg mL^{-1}) was achieved against double distilled water using scan mode by UV/Vis spectrophotometer to get zero order spectrum. By analyzing the obtained absorption spectrum was observed maximum absorbance at 262 nm.

Preparation of stock standard solution

Selection of solvent was based on solubility of drug in solvent system. Bupivacaine hydrochloride substance can be soluble in water and freely soluble in alcohol. So, as solvent for analysis was selected double distilled water.

To prepared stock standard solution an accurately weighed quantity of bupivacaine hydrochloride was transferred into 100 ml volumetric flask to get the final concentrations of 2 mg mL^{-1}. After prepared, the stock standard solution was stored at low temperature.

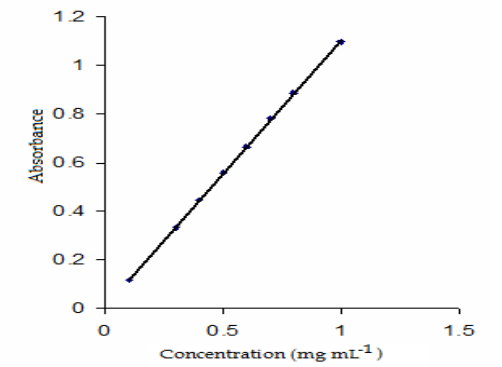

Figure 2. Calibration curve for bupivacaine hydrochloride

Preparation of sample solutions

It was prepared solution with double distilled water from pharmaceutical preparation that contains 5 mg mL^{-1} bupivacaine hydrochloride. 5 ml (1 ampoule) was transferred quantitatively with distilled water in a 50 ml volumetric flask which was filled to the mark to get the sample solution.

Results and Discussions

Linearity

From the stock solution were made dilutions to obtain solutions over the concentration range 0.1-1 mg mL^{-1}. All standard solutions were prepared in double distilled water.

Three determinations for each concentration were made and a mean value of the absorbance read at 262 nm was calculated (Table 1). The calibration curve obtained by plotting the mean values of the absorbance of bupivacaine hydrochloride *vs* bupivacaine hydrochloride concentrations (mg mL^{-1}) is presented in Figure 2. The statistical interpretation of the data obtained led us to the following results, presented in Table 2.

Table 2. Statistical data regarding bupivacaine hydrochloride determination

Statistical data	Bupivacaine hydrochloride
Person coefficient (r^2)	0.99981
Standard Error	0.004735
Intercept	0.00796
Slope	1.0949
Limit of detection	0.014269
Limit of quantification	0.04324

Repetability (system precision)

The system precision was evaluated at concentrations of 0.6 mg mL^{-1} in 9 replicates, we registrated the absorbances and than we calculated average, standard deviation and % RSD.

Experimental data of the absorbances relating to the precision of the system are shown in Table 3.

Table 3. Experimental data of the absorbances relating to the precision of the system

No	Absorbance
1.	0.667
2.	0.669
3.	0.672
4.	0.675

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5.	0.659
6.	0.66
7.	0.664
8.	0.67
9.	0.671
Average	0.6674
SD	0.005456902
% RSD	0.817581432

Relative standard deviation (RSD) was found to be 0,8175 % which is lower than the maximum 2 % proposed by the european standards, therefore the system is considered to be precise.

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Table 4. Calculated concentrations and the recovery of the method in the same day

9.00 a.m.			12.00 a.m.			15.00 p.m.		
Absorbance	**Calcd. concentration**	**% Recovery**	**Absorbance**	**Calcd. concentration**	**% Recovery**	**Absorbance**	**Calcd. concentration**	**% Recovery**
0.443	0.39731	99.3296	0.446	0.40005	100.0146	0.441	0.39549	98.8729
0.448	0.40188	100.4713	0.448	0.40188	100.4712	0.444	0.39823	99.55796
0.442	0.39640	99.10132	0.447	0.40097	100.2429	0.442	0.39640	99.10132
0.671	0.60554	100.9249	0.659	0.59458	99.09827	0.665	0.60006	100.01156
0.669	0.60372	100.6204	0.668	0.60281	100.4682	0.659	0.594589	99.0982
0.677	0.61102	101.8382	0.66	0.59550	99.2504	0.663	0.598242	99.7071
0.886	0.80190	100.2384	0.884	0.80008	100.0100	0.886	0.801906	100.2383
0.89	0.80556	100.695	0.887	0.80282	100.3525	0.882	0.798253	99.7817
0.887	0.80282	100.3525	0.887	0.80282	100.3525	0.883	0.799167	99.8958
	Average	100.3968		Average	100.02898		Average	99.5850
	SD	0.817388		SD	0.514356		SD	0.46605
	% RSD	0.814157		% RSD	0.514207		% RSD	0.46799

Table 5. Recovery values of the method in 3 consecutive days

	% RSD		**% RSD**		**% RSD**
Day 1	0.82	Day 2	0.96	Day 3	1.01

Table 6. Bupivacaine hydrochloride concentrations found in samples

	Day 1		**Day 2**		**Day 3**	
Concentration declared/ampoule mg mL^{-1}	**Concentration found/ampoule mg mL^{-1}**	**% Recovery**	**Concentration found/ampoule mg mL^{-1}**	**% Recovery**	**Concentration found/ampoule mg mL^{-1}**	**% Recovery**
5 mg mL^{-1}	5.0937	101.8759	5.01433	100.2867	5.0627	101.2549
	4.9905	99.81186	4.9869	99.7387	5.0700	101.401
	5.0965	101.9306	5.0508	101.0174	4.9686	99.3734
	5.0636	101.2731	4.9412	98.8255	5.0371	100.7434
	5.0682	101.3644	4.9321	98.6428	5.0326	100.6521
	4.9759	99.5196	4.9960	99.9214	5.0179	100.3598
	5.0362	100.7251	5.0417	100.8347	4.9695	99.3917
	5.0344	100.6886	4.9960	99.9214	4.9869	99.7388
	5.0316	100.6338	5.0691	101.3827	5.03991	100.7982
Average	5.04346	100.86925	5.0031	100.0635	5.0182	100.4126
SD	0.04182	0.83642	0.0467	0.9348	0.0378	0.7566
% RSD	0.8292	0.82921	0.9342	0.9342	0.7539	0.7535

Method precision and accuracy

Method precision was investigated on a range of 20 % compared to a reference concentration, grouped on a number of 3 determinations in 3 concentration levels. Studies were performed the same day at different times (9.00 a.m., 12.00 a.m. and 15.00 p.m.) and in 3 consecutive days (day 1, 2 and 3). From the absorbances obtained, we determined the concentration calculated, % recovery, average, standard deviation and % RSD. Table 4 and 5 shows the results of recovery tests in the same day at different times and in consecutive days.

The mean recovery of the tests made in the same day was in the range 99,58 - 100,39 %, with % RSD = 0.46 – 0.81.

The RSD values in 3 different days were in the range 0.82 – 1.01. In both cases (in the same days and in 3 consecutive days) the RSD values are lower than maximum 5 % proposed by the european standards, therefore the method is accurate.

Application on pharmaceutical forms

We investigated our samples (from pharmacy) after dilution to obtain 0.5 mg mL^{-1} bupivacaine hydrochloride, in 3 consecutive days, in 9 replicates each day. The results are presented in Table 6. The results obtained in the 3 days comply with the limits stipulated by the regulations.

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Conclusions

This study presents the development and validation of a simple, cheap, reliable, accurate and precise spectrophotometric method for the determination of bupivacaine hydrochloride. The results obtained showed that the proposed method for the quantification of bupivacaine hydrochloride comply with all the validation parameters and has been successfully applied in the determination of substance in pharmaceutical formulations.

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Bupivacaine HCl 0.5% 5 mg mL^{-1} injection, USP 50 ml multi dose vial, FDA, Official FDA information, side effects and uses.

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