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Simultaneous determination of paracetamol and lidocaine hydrochloride in detamol injection using RP-HPLC

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ABSTRACT: The current study describes the simultaneous determination of paracetamol and lidocaine hydrochloride in Detamol injection using RP-HPLC with fluorescent light detection (FLD). Samples elution was done through the HPLC column (C18-XDB, 5µm, 4.6 × 150 mm) with isocratic mobile phase- a mixture of water, acetonitrile and tetrahydrofuran (90:5:5, v/v/v) at a flow rate of 1.0 ml/min using FLD detector (250 nm excitation and 410 nm emission). The analytical method was linear for both the analytes, over the investigated range with the correlation coefficient (R^2) = 0.993 - 0.995. The limit of detection (LOD) and limit of quantification (LOQ) were as: paracetamol 0.894 µg/ml, 2.98 µg/ml, respectively, and lidocaine HCl 0.53µg/ml, 1.78µg/ml, respectively. The analytical method was reliable, precise and accurate (RSD < 5%). The developed method was successfully applied to quantify paracetamol and lidocaine HCl in Detamol injection. The results of current study indicated that developed method is linear, precise, accurate and sensitive, hence may be used for quality control purposes.

KEYWORDS: Simultaneous determination; paracetamol; lidocaine HCl; validation; quantification; derivitization; ninhydrin

1. INTRODUCTION

Detamol injection is a combination of paracetamol (chemically acetaminophen)- a non-opiate analgesic and anti-pyretic drug with lidocaine HCl- an amide-type local anesthetic and anti-arrhythmic agent which is indicated for management of mild to moderate pains e.g., in cases of migraine, headache, myalgia, neuralgia, toothache, algomenorrhea; trauma and burns etc. and pyrexia associated with infectious and inflammatory diseases. The positive drug-drug interaction between paracetamol and lidocaine HCl where the later potentiates the effects of the former, favours the patient by minimizing the switching to opiates for analgesia [1]. Literature review also indicates use of codeine, lidocaine HCl and paracetamol, in combination, for producing analgesia in prostate gland biopsy [2].

Diverse types of methods have been reported in literature for the estimation of paracetamol and lidocaine HCl, either separately or in combinations with other drugs. HPLC method was reported for simultaneous quantification of paracetamol, diclofenac sodium and methocarbamol [3]. A handsome literature is available on stability-indicating liquid chromatographic methods for quantification of paracetamol in combinations with tramadol hydrochloride and domperidone, guaiphenesin, sodium benzoate and oxomemazine in cough syrup and caffeine and dipyrone [4-6]. The literature also indicated the use of HPLC coupled UV and radioactivity detectors for determination of acetaminophen and its metabolites in human samples [7]. Multiple pulse amperometric detection was also reported for determination of ascorbic acid and acetaminophen [8]. The identification and determination of lidocaine HCl is reported in literature, using an acidic iodoplatinate reagent and various techniques such as LC-Tandem MS, HPLC with diode array detection and GCMS [9, 10]. HPTLC method has been reported in literature for estimation of lidocaine HCl and phenylephrine HCl [11].

There is no analytical method reported in literature for quantification of lidocaine HCl and paracetamol simultaneously. Hence, in current study, a reversed phase HPLC method was developed and validated for quantification of paracetamol and lidocaine HCl, found in Detamol injection.

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2. RESULTS AND DISCUSSION

2.1. Development of the method

Primarily, efforts were made to develop a HPLC method by pre-column derivatization of lidocaine HCl in such a way that this does not affect UV behaviour of paracetamol. For this purpose, ninhydrin reagent was utilized and it was believed that amino group in lidocaine may react with the reagent to produce Ruhmann purple color, an indicative of the positive result. Unfortunately, the attempt remained unsuccessful and a major factor contributing to false result was the structure of the drug. Since Schiff base formation, as a result of condensation of the reagent with the drug, an alpha proton is required in amino compounds, which is only possible in ammonia and primary amines. It is evident from the structure of the lidocaine HCl that it contains tertiary amino group, which being adjacent to a tertiary carbon cannot be detected by the ninhydrin reagent. Moreover, the formation of color is also affected by various factors such as concentration of analyte, strength of ninhydin in the reagent, temperature and pH (acidic environment is favorable). Therefore, it is concluded from the results that pre-column derivatization of lidocaine HCl with ninhydrin reagent is not possible.

The benzene chromophore of lidocaine was extended by additive effect of the carbonyl group. Moreover, under basic conditions, the lone pairs of the nitrogen (amino group as an auxochrome) result in bathochromic shift and hyperchromism. However, the drug as a hydrochloride salt is acidic and under such condition, amino groups are protonated and cause hypochromisim, which may compromise sensitivity of UV detector. Therefore, it was aimed to detect the drug by FLD, which was operated at various excitation and emission wavelengths. It was found that the response of the FLD was maximum at excitation wavelength of 250 nm and 410 emission wavelengths. Therefore, the method was developed using these wavelengths.

For elution, various compositions of mobile phases, mixtures of water and methanol in different proportions (50:50, 60:40, 80:20, v/v) and mixtures of water and acetonitrile (50:50, 60:40, 80:20, v/v), were tried using C18 column. The optimum resolution was achieved using mobile phase, comprising of water and acetonitrile (90:10, v/v). However, the tetrahydrofuran was added in mobile phase, to achieve peak symmetry. The peaks of paracetamol and lidocaine hydrochloride appeared at retention time of 3.103 min and 3.989 min, respectively (Figure 1).

These results showed that peaks of both the analytes were fully separated and symmetrical. The system suitability parameters are given in Table 1.

The results were within the specified limits which indicated that the chromatographic system and conditions were suitable to be used for quantitative purposes. The method was then validated for a number of parameters, which are given as follows:

2.2. Validation of the method

The HPLC method was validated using the ICH guidelines. [12]

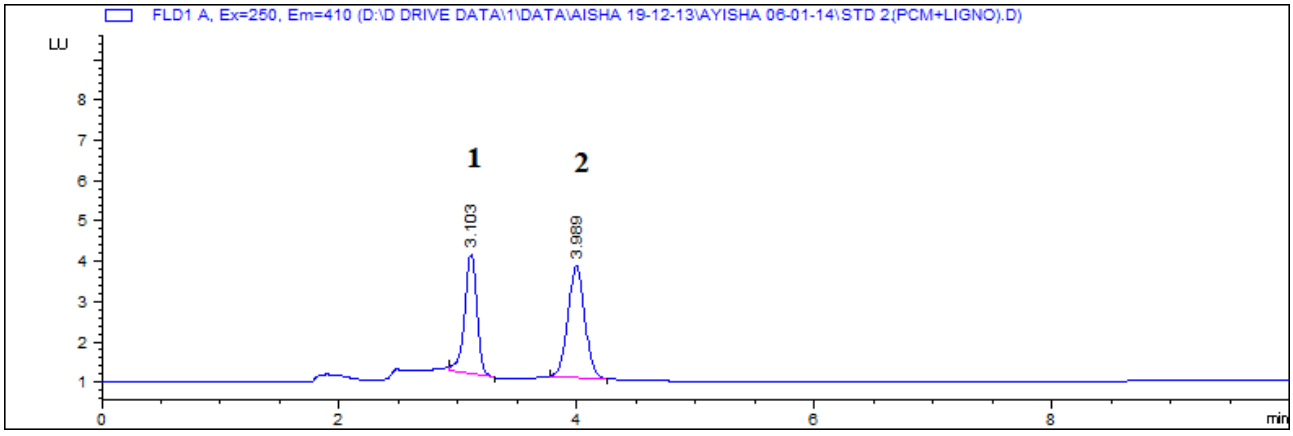

Figure 1. Chromatogram of mix standard solution of paracetamol (100 µg/mL) and lidocaine hydrochloride (100 µg/mL), 1 (paracetamol); 2 (lidocaine hydrochloride)

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Table 1. System suitability parameters calculated from chromatogram of paracetamol and lidocaine hydrochloride

Parameters	Paracetamol	Lidocaine hydrochloride	Reference values
Capacity(retention) factor k´	2.33	3.284	K ≥ 2
Peak asymmetry/Tailing factor As	1.0	1.0	T ≤ 2
Number of theoretical plates N	13608.14	10816	N > 2000
Height equivalent to theoretical plate	11.02	13.86	The smaller the value, higher the efficiency of the column

2.2.1. Linearity

The method was linear for both the analytes over the range investigated (20.0-100.0 µg/ml). The linear regression equation for paracetamol was found to be Y=0.315x + 0.56 with (R^2 =0.9907), while the equation of lidocaine HCl was found to be Y=0.3075x + 0.23 with R^2 =0.9989.

2.2.2. Sensitivity

The LOD (S/N, 3: 1) and LOQ (S/N, 10: 1) values of paracetamol were calculated out to be 0.894 and 2.98µg/mL, respectively (Table 2). The LOD and LOQ values of lidocaine hydrochloride were calculated out to be 0.53 and 1.78µg/mL, respectively (Table 2). The sensitivity values of paracetamol, determined in the present study, were slightly lower than the two studies [4,11], and slightly higher than that reported earlier [13]. Likewise, the LOD and LOQ values of lidocaine HCl, determined in current study were lesser than reported previously [14-16], which indicated the higher sensitivity of the method.

2.2.3. Specificity and recovery

The method was found to be specific for both the analytes, because the response of each of the analytes was not affected by the other. Moreover, the recovery of the method was found to be in range 95.6 to 106.9% for paracetamol and 93.0 to 103.4% for lidocaine hydrochloride (Table 3) which indicated reliability and specificity of the method.

2.2.4. Intraday and inter-day analysis

The results of intra and inter-day analysis are shown in Table 3, which showed reproducibility and repeatability of method.

2.2.5. Robustness

The developed method was robust as accuracy of assay was unaffected by small variation in mobile phase composition and column temperature.

2.3 Quantification of paracetamol and lidocaine HCl in Detamol injection

The current method was used for quantification of paracetamol and lidocaine HCl in Detamol injection. The percentage contents were found to be 100.75 % and 104.08% for paracetamol and lidocaine HCl, respectively (Table 4).

Table 2. Lowest limit of detection (LOD) and lowest limit of quantification (LOQ) calculation for paracetamol and lidocaine hydrochloride by HPLC analysis

Analyte	Concentration range (µg/mL)	Mean slope	Intercept (S.D)	LOD (µg/ml)	LOQ (µg/ml)
Paracetamol	20-100	0.003	0.000894	0.894	2.98
Lidocaine hydrochloride	20-100	0.0196	0.003493	0.53	1.78

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Table 3. Recovery, Intraday and inter-day and accuracy and precision of assay of paracetamol and lidocaine hydrochloride by HPLC analysis (n=6)

Analyte	Concentration (µg/mL)	Recovery	Intraday assay		Interday assay	
			Accuracy	Precision(RSD)	Accuracy	Precision(RSD)
Paracetamol	40	106.94±0.0015	107.5	4.36	107.08	2.85
	60	106.77±0.0057	98.70	4.84	96.85	4.99
	80	95.625±0.01	99.27	3.39	101.87	3.24
Lidocaine hydrochloride	40	93±0.0057	93.85	1.33	96.32	1.29
	60	103.4±0.05	109.96	4.38	101.78	4.76
	80	93.5±0.05	100.86	3.53	108.2	3.63

Table 4. Percentage purity of paracetamol and lidocaine hydrochloride in injection by HPLC (n=3)

Drug	Percentage contents
Paracetamol	100.75%
Lidocaine hydrochloride	104.8%

The 2 ml injection of Detamol contains 300 mg paracetamol and 20 mg lidocaine HCl. This difference in the contents of both the analytes resulted in a chromatogram having a very high peak of the paracetamol and a very small peak of lidocaine HCl. Therefore, a standard addition method was adopted for lidocaine HCl to get the equal response of both the drugs. Therefore, 0.1 ml of the sample diluted to 1.0 ml gave a signal equivalent to 0.25 ml of the standard diluted to 1.0 ml. This volume was then used to determine the amount of lidocaine HCl in the injection. On the other hand, paracetamol was determined using linear regression equation of standard calibration curve. The chromatogram of the Detamol injection is given in Figure 2. Blank chromatogram of both analytes shown in Figure 3.

The aim of our study is simultaneous quantification of paracetamol and lidocaine hydrochloride using simple HPLC method. However, this study will be helpful in stability and compatibility studies of paracetamol and lignocaine hydrochloride with locally available drip sets and microburettes used in hospitals.

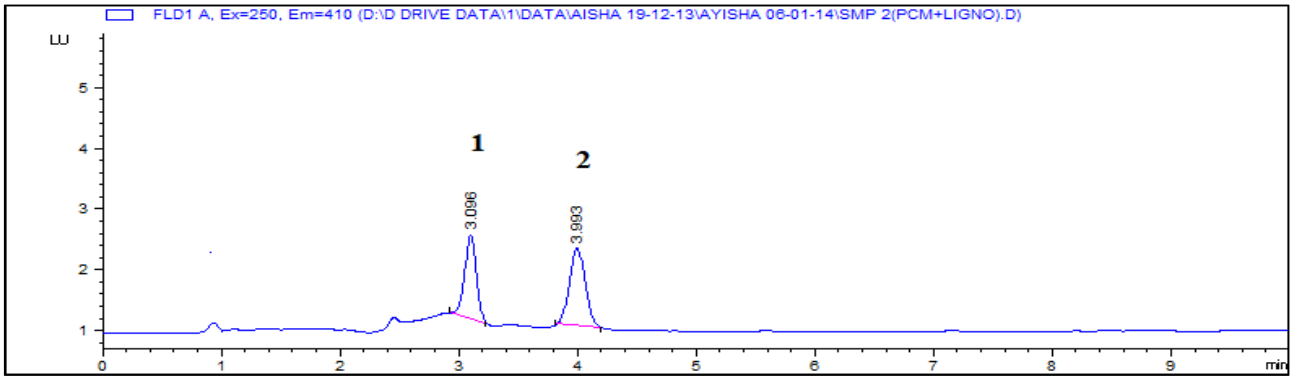

Figure 2. Chromatogram of paracetamol injection containing lidocaine hydrochloride, 1 (paracetamol 50 µg/mL); 2(lidocaine hydrochloride 50 µg/mL)

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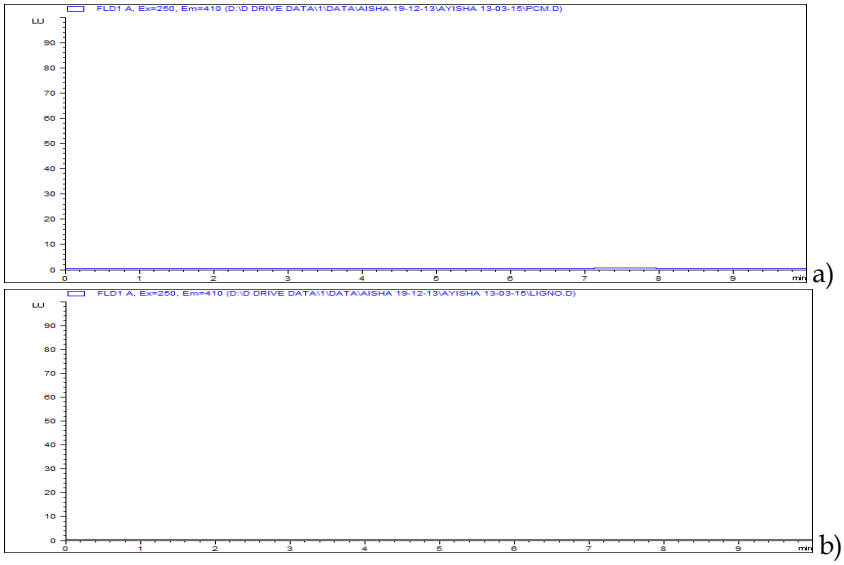

Figure 3. HPLC chromatogram of blank for both analytes, a) Paracetamol, b) Lidocaine hydrochloride

As discussed in the introduction section, there are numerous methods reported for HPLC determination of paracetamol and lidocaine hydrochloride individually but there is no method reported for the simultaneous quantification of both analytes. The dosage form available in market containing both these analytes can be easily quantified using this simple method. The mobile phase was polar in nature, hence devoid of expensive ion pairing agents. Furthermore, this detection is easy as compared to those where pre-column derivatization has been used [17]. Elimination of the derivatization step makes this method suitable, less laborious and less expensive.

The solution containing paracetamol and lidocaine hydrochloride each having concentration 50 µg/ml was prepared in mobile phase, and was analyzed in HPLC at 0, 30, 60, 120 minutes, 24 hours and 48 hours, it was determined whether there was any change in the values. The results obtained are shown in Table 5.

Table 5. Stability test results for 50 µg/mL concentration for both analytes

Time	Paracetamol		Lidocaine hydrochloride	
	Peak area	Concentration	Peak area	Concentration
0 min	16.31	50.0	15.61	50.01
30 min	16.30	49.96	15.60	49.98
60 min	16.20	49.65	15.57	49.88
120 min	16.1	49.33	15.54	49.78
24 h	15.87	48.60	15.1	48.35
48 h	15.4	47.11	14.85	47.54
Average (Mean±SD)	16.03±0.348	49.11±1.01	15.37±0.323	49.26±0.959

3. CONCLUSION

This is an analytical method reported for the first time for simultaneous quantification of paracetamol and lidocaine HCl using RP-HPLC with florescent light detection FLD). The method is simple, accurate, reproducible and repeatable.

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4. MATERIALS AND METHODS

4.1 Chemicals

Paracetamol and lidocaine HCl were gifted by the Remington Pharmaceuticals, Lahore, Pakistan. Detamol injection (Thai PD, Chemicals), HPLC grade solvents such as methanol, acetonitrile, tetrahydrofuran and sodium hydroxide (Merck, Germany) were purchased from market.

4.2 Instruments

The analysis was carried out using HPLC system (series 1200, Agilent Technologies, Germany), having isocratic pump, auto-sampler, thermostatically controlled column oven, diode array detector (DAD) and fluorescent light detector (FLD). The equipment was having operating software, Chemstation for LC and LC-MS, version A. 08. 03.

4.3 Standard solutions preparation

Paracetamol (1.0 mg/ml) and lidocaine HCl (1.0 mg/ml) standard stock solutions were prepared in 0.1N NaOH. Two hundred microliters of both the stock solutions were mixed with volume make up to 1.0 ml with methanol to get a mixed standard stock solution of concentration 200 µg/ml, of each analyte.

Series of mixed working standard solutions of each analyte were prepared by dilution of mixed stock solution with methanol to give concentration 5.0, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 µg/ml with filtration of solutions via 0.45 µm polytetrafluoroethylene syringe filters and filled in HPLC vials.

4.4. Chromatographic conditions

Sample elution (20 µl) was done via HPLC column (C18 XDB, 5 micrometer, 4.6 × 150 mm) at 1 ml/min flow rate, with mobile phase (water, acetonitrile and tetrahydrofuran (90 : 5 : 5, v/v/v) elution in isocratic mode having column temperature of 25°C and detection via fluorescence detector (excitation wavelength 250 nm and 410 nm emission wavelength).The chromatogram obtained was used to determine system suitability.

4.5. System suitability

The system suitability was ensured by determining number of theoretical plates (N), height equivalent to theoretical plate (HETP), capacity factor (k´), tailing factor and peak asymmetry. Dead volume was calculated using following formula:

Dead volume (V^0)= dead time (t^0)× flow rate of mobile phase (F^o)

where, dead time (t^0) is the time when the mobile phase (solvent) enters the column.

4.6. Method validation

This was carried out by determining the parameters, which are given as follows:

4.6.1. Linearity

The standard solutions of each analyte (20.0 to 100.0 µg/ml) were analyzed in triplicates and response of each of the concentrations was plotted against peak height. Linearity was assessed by observation of calibration curve and data points correlation was evaluated by determining R^2 (correlation coefficient).

4.6.2. Sensitivity: Limit of detection (LOD) and limit of quantification

Sensitivity of method was determined statistically by LOD and LOQ, at a signal to noise (S/N) ratio 3: 1 and 10: 1, respectively. Five standard solutions (20.0 to 100.00 µg/ml) were analyzed in quintuplicate, to construct curves and the resulting data was used for statistical determination of LOD and LOQ.

$$\mathrm{LOD} = \frac{3\ \mathrm{SD}}{\mathrm{S}}$$

$$\mathrm{LOQ} = \frac{10\ \mathrm{SD}}{\mathrm{S}}$$

4.6.3. Recovery, Intraday and Interday analysis

For recovery, triplicate analysis of standard solutions (40, 60 and 80 µg/ml) was carried out with concentration recovered determined from the calibration curve. Recovery was assessed by dividing the determined value with the true value, and expressed in percentage ± SD.

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The three standard solutions were analyzed six times a day for intraday analysis and once a day for six days consecutively for interday analysis.

4.7 Determination of paracetamol and lidocaine HCl in Detamol injection

4.7.1. Preparation of samples

The Detamol injection having 150 mg/mL paracetamol and 10.0 mg/ml lidocaine HCl was diluted with HPLC grade methanol in order to get a sample stock containing 7.5 mg/ml paracetamol and 0.5 mg/ml lidocaine HCl. One hundred microliters of the stock solution were diluted to 1.0 ml to get the working sample solution for paracetamol. For lidocaine HCl, 100 µl of the stock solution was added in six vials and a standard solution of lidocaine HCl (200 µg/ml) was added in these vials as 0.0, 25.0, 75.0, 175.0, 275.0 and 375.0 µl, and made the volume 1.0 ml. The working sample solutions were filtered like the standard solutions and filled in HPLC vials.

The solution containing paracetamol and lidocaine hydrochloride each having concentration 50 µg/ml was prepared in mobile phase, and was analyzed in HPLC at 0, 30, 60, 120 minutes, 24 hours and 48 hours and it was determined whether there was any change in the values.

4.7.2. HPLC analysis

The working sample/standard solution (20 µl) was eluted at the chromatographic conditions mentioned above. The peaks of both the analytes were identified by comparison of their retention times to that of the standards. The paracetamol was quantified by external standard method, whereas lidocaine HCl was quantified by the standard addition method, using linear regression equation, obtained from standard calibration curves.

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