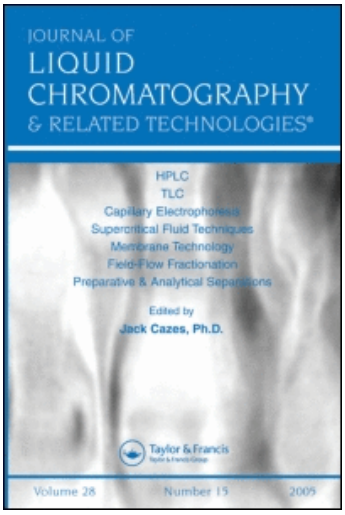

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DETERMINATION OF HYOSCINE N-BUTYL-BROMIDE, LIDOCAINE HYDROCHLORIDE, AND PARACETAMOL IN INJECTION FORMS USING SOLID-PHASE EXTRACTION, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, AND UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

A solid phase extraction procedure using strong cation exchange (SCX, benzenesulfonic acid) cartridges followed by a reversed-phase HPLC assay was applied to the analysis of hyoscine n-butylbromide and lidocaine hydrochloride in injection forms. The chromatographic separation was performed on a BDS C-18 column. The mobile phase consisted of a mixture of acetonitrile : ammonium acetate 0.2M, (30:70, v/v) pumped at a flow rate 1.2 mL/min. The UV detector was operated at 254 nm. A UV-Vis spectrophotometric method was also developed for the determination of paracetamol in the injection forms. The method consists of subsequent dilution of the injection forms and measure of the absorbance value at 242.7 nm. Relative standard deviation was less than 0.95% for HPLC and less than 0.78% for the

spectrophotometric method. Detection limits were 1.05, 0.96 and 0.67 μg/mL for hyoscine n-butylbromide, lidocaine hydrochloride and paracetamol, respectively.

INTRODUCTION

Hyoscine n-butylbromide, (-)-(1S,3s,5R,6R,7S)-8-butyl-6,7-epoxy-3-[(S)-tropoyl-oxy]tropanium bromide, is a quaternary ammonium salt of hyoscine. It is an anticholinergic drug which has been used to relieve detrusor muscle spasms.[1] It is poorly absorbed from the gastrointestinal tract and does not readily pass the blood-brain barrier.[2,3] Paracetamol,[4] N-acetyl-p-aminophenol, is a widely used mild analgesic and antipyretic drug.[5] The combination of these two drugs in injection forms is used effectively in the treatment of spastic gastrointestinal disorders. Lidocaine hydrochloride,[6] 2-diethylamino-2',6'-dimethylacetanilide hydrochloride, is a local anesthetic of the amide type used as an additive in this injection form to relieve pain during the injection.

A variety of methods have been reported for the determination of hyoscine n-butylbromide such as potentiometric titration,[7] conventional ultraviolet spectrophotometry[8] and high performance liquid chromatography.[9,10] Among the methods for paracetamol assay are UV spectrophotometry,[11] derivative spectrophotometry,[12,13] spectrofluorimetry,[14] GC,[15] and HPLC.[16-19] Derivative UV-Visible spectrophotometric[20] and high performance liquid chromatographic[21] methods have also been described for the determination of lidocaine hydrochloride. No references were found to the simultaneous determination of hyoscine n-butylbromide, lidocaine hydrochloride, and paracetamol in a ternary mixture.

The present work investigates the simultaneous determination of hyoscine n-butylbromide and lidocaine hydrochloride in injectable solution using a solid-phase extraction method followed by HPLC analysis. A simple and rapid UV-visible spectrophotometric method is also developed, allowing the quantitative determination of paracetamol in the same injection form.

MATERIALS AND METHODS

Apparatus

The high performance liquid chromatographic system consisted of a GBC Model LC1126 pump and a Rheodyne Model 7725i injector with a 20-μL loop, which were coupled to a GBC Model LC1210 UV-Vis detector with a 8-μL

flow cell operated at 254 nm. The chromatographic apparatus is controlled by a WinChrom software package: ChemWin, version 1.2. UV-visible spectra were recorded on a Perkin-Elmer, Model Lambda 7, double-beam UV-visible spectrophotometer. A pH meter (Metrohm, model 654 Herisau) was used for pH measurements.

Materials

Solvents were of HPLC grade and were purchased from Lab-Scan Science Ltd., Ireland. Ammonium acetate (pro analysi), potassium dihydrogen phosphate, and hydrochloric acid (analytical reagent grade) were purchased from E. Merck Ltd., Germany. Water was deionised and further purified by means of a Milli-Q Plus Water Purification System, Millipore Ltd. Strong cation exchange, SCX (*benzenesulfonic acid*), cartridges were obtained from Varian SPP (USA). Hyoscine n-butylbromide, paracetamol, and lidocaine hydrochloride of pharmaceutical purity grade were kindly provided by Uni-Pharma Hellas A. E., Athens, Greece and were used without any further purification. SPASMO-APOTEL® is a 4-mL injectable solution containing 150 mg/mL of paracetamol, 5 mg/mL of hyoscine n-butylbromide, 5 mg/mL of lidocaine hydrochloride, different excipients, and suitable mixture solvents.

Methods

Chromatographic separation was performed on a reversed phase BDS C-18 column (250 × 4.6 mm i.d., 5 μm particle size) (Shandon Scientific Ltd, Chesire, U.K.). The mobile phase, acetonitrile : ammonium acetate 0.2 M (30 : 70, v/v), was filtered through a 0.45 μm Millipore filter and degassed under vacuum prior to use. All chromatographic experiments were carried out at a flow rate 1.2 mL/min.

UV-visible spectra were recorded over a wavelength range 210 to 310 nm and the absorbance value at 242.7 nm was measured for the determination of paracetamol. All spectrophotometric measurements were carried out in 1 cm matched quartz cells. The optimized operating conditions for spectrophotometric measurements were scan speed 60 nm min^{-1}, response 0.5 s and slit-width 2 nm.

Stock Standard Solutions

Stock standard solutions of hyoscine n-butylbromide, HBB, (1.0 mg/mL) and lidocaine hydrochloride, LID, (1.0 mg/mL) were prepared by dissolving the

compounds in water. Mixed working standard solutions containing 100 to 350 μg/mL of HBB and 100 to 350 μg/mL of LID in a ratio 1:1, were also prepared by dilution of the above mentioned stock solutions in water. Stock standard solution of paracetamol (1.0 mg/mL) was prepared by dissolving the compound in methanol. The stock and working standard solutions were stored in the dark under refrigeration and was found to be stable for several weeks.

Sample Preparation for Chromatographic Procedure

A 1.25 mL aliquot of the injection solution was transferred to a 25-mL volumetric flask and diluted to volume with water. A 0.6-mL aliquot of this solution was diluted to 1-mL with phosphate buffer solution (pH =4.5). The resulting solution was applied to a Bond-Elut SCX cartridge (Strong Cation exchange), which was preconditioned with 1 mL of water and 1 mL of phosphate buffer solution (pH 4.5). The column was washed with 2 × 1 mL phosphate buffer (pH 4.5) and 1 × 1 mL water. The absorbed solutes, HBB and LID, were then slowly eluted from the column using 2 × 1 mL of methanol -1.0 N hydrochloric acid (70:30, v/v).

Sample Preparation for Spectrophotometric Procedure

An aliquot of the injection solution, equivalent to about 187.5 mg of paracetamol, was subsequently diluted in water to give a solution containing 12.0 μg/mL of paracetamol.

Calibration Procedure

A series of mixed standard aqueous solutions of HBB and LID in a ratio 1:1, was prepared by the appropriate dilution of the above mentioned mixed working standard solutions to 2 mL of a mixture of methanol - 1.0 M HCl (70:30, v/v). The concentration range tested was 30 to 105 μg/mL for each one of the compounds. Peak area measurements of each compound were plotted against the corresponding concentration to obtain the calibration graphs.

In order to determine HBB and LID in injection forms, two other calibration curves were constructed by assaying mixed samples containing 60 to 210 μg/mL of HBB and LID in a ratio 1:1. These samples were prepared by the addition of the appropriate dilution of the mixed working standard solutions in 1 mL of phosphate buffer (10^{-1} M KH_2PO_4, pH = 4.5) and were subjected to the solid phase extraction procedure prior to the chromatographic assay.

A series of standard aqueous solutions containing 3 to 18 μg/mL of paracetamol were assayed spectrophotometrically. Moreover, the specificity of the spectrophotometric method in the determination of paracetamol was also investigated by analyzing another series of mixed standard aqueous solutions containing 3 to 18 μg/mL of paracetamol and 0.1 to 0.6 μg/mL of HBB and LID, in a ratio 1:1. The absorbance values at 242.7 nm, $A_{242.7}$, were measured for the determination of paracetamol.

The over-all precision of the chromatographic assay was evaluated by analyzing three series of mixed standard solutions containing 60.0, 75.0, and 90.0 μg/mL respectively of a binary mixture of HBB and LID in a ratio 1:1 with eight replicates. The precision and accuracy of the spectrophotometric method was also evaluated by assaying three series of mixed standard solutions containing paracetamol, HBB, and LID in a ratio 30:1:1. The concentrations of paracetamol in these three series were 3, 9, and 18 μg/mL, respectively.

The precision of each method was based on the calculation of the relative standard deviation (%RSD). An indication of the accuracy was based on the relative percentage error of the samples (E_r%).

RESULTS AND DISCUSSION

Chromatographic Characteristics

For the simultaneous determination of HBB and LID the chromatographic conditions described above were used. Under these chromatographic conditions paracetamol was eluted at 2.5 min. Paracetamol elution, due to its high molar absorptivity and high concentration in the analyzed injection forms, overloads the chromatographic column and serious overlap to the chromatographic peak of HBB, which was eluted at 4.73 min. is observed. This prompted us to develop a solid phase extraction technique[22] which allowed the isolation of HBB and LID from paracetamol.

A thorough investigation was conducted in order to choose the optimum conditions for the solid phase extraction technique. For the clean-up of the analyzed injection forms a SCX packing material (benzenesulfonic acid) was preferred. At pH 4.5 (phosphate buffer solution), the protonated lidocaine (basic substance, pKa 7.9) and the positively charged hyoscine n-butylbromide (quaternary ammonium salt) was retained by the sorbent, while the uncharged paracetamol (weak acid, pKa 9.5) passed through the column.

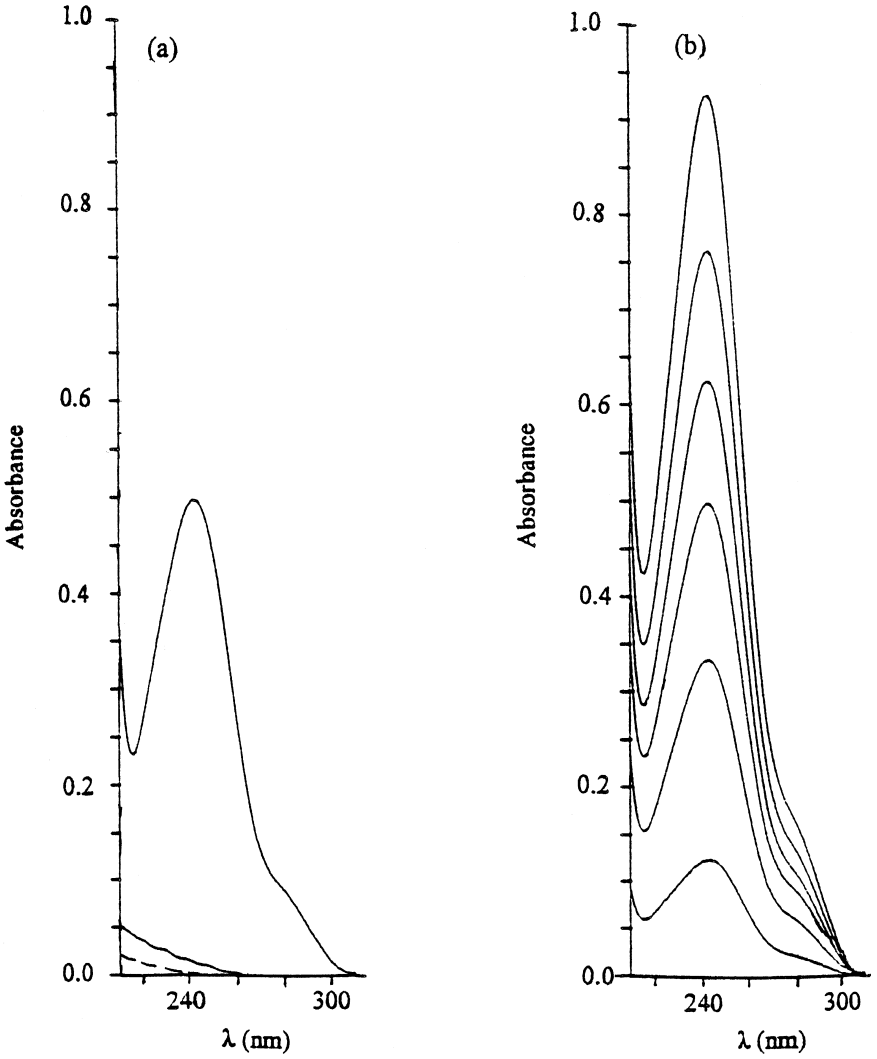

Figure 1. a) Representative chromatogram obtained from the analysis of a blank sample, b) representative chromatogram obtained from the analysis of an injection sample. Hyoscine n-butylbromide and lidocaine hydrochloride were eluted at 4.73 and 13.60 min, while two of the excipients were also eluted at 5.87 and 19.5 min. Chromatographic conditions: Reversed-phase HPLC on a C-18 BDS column; mobile phase: acetonitrile : 0.2 M ammonium acetate (30:70, v/v) and a UV detector set at 254 nm.

This procedure also allows elimination of neutral excipients which, in large excess, could interfere with the chromatographic determination. After appropriate washing to remove the majority of these excipients, HBB and LID were eluted using an acidic solvent system.

Typical chromatograms of a blank and an injection sample obtained using the above mentioned SPE technique is displayed in Figure 1(a) and 1(b), respectively. HBB and LID were eluted at 4.73 and 13.60 min, respectively. Two of the excipients were also eluted at 5.87 and 19.47 min, respectively and were well separated for HBB and LID. It is obvious that a very good separation of these substances was accomplished, under the selected chromatographic conditions. This implies that using the HPLC method, HBB and LID could be determined accurately without any interference from the excipients of the injection form.

Spectral Characteristics

The UV-visible absorption spectra of 9.0 μg/mL of paracetamol, 0.3 μg/mL of HBB and 0.3 μg/mL of LID are presented in Figure 2(a). The working wavelength region was chosen to be between 210 and 310 nm. HBB and LID, does not show any significant absorption over the selected wavelength region. Therefore, the absorbance value at 242.7 nm could be effectively used for the quantitative determination of paracetamol in presence of HBB and LID, Figure 2(b) shows a typical set of UV-visible absorption spectra of mixed aqueous solutions, containing 3 to 18 μg/mL of paracetamol and 0.1 to 0.6 μg/mL of HBB and LID in a ratio 1:1.

Evaluation of the HPLC Method

Linearity and reproducibility

Under the experimental conditions described above, linear relationship between the HPLC signal of HBB and LID and their concentration were observed, as shown by the equations presented in Table 1.

The efficiency of the solid phase extraction procedure was determined by calculating the ratio of the slopes of the regression equations obtained from extracted mixed samples to those for unextracted standard aqueous solutions, containing binary mixture of HBB and LID, in a ratio 1:1. The absolute recovery of the method was found to be 91.9% for HBB and 88.8% for LID.

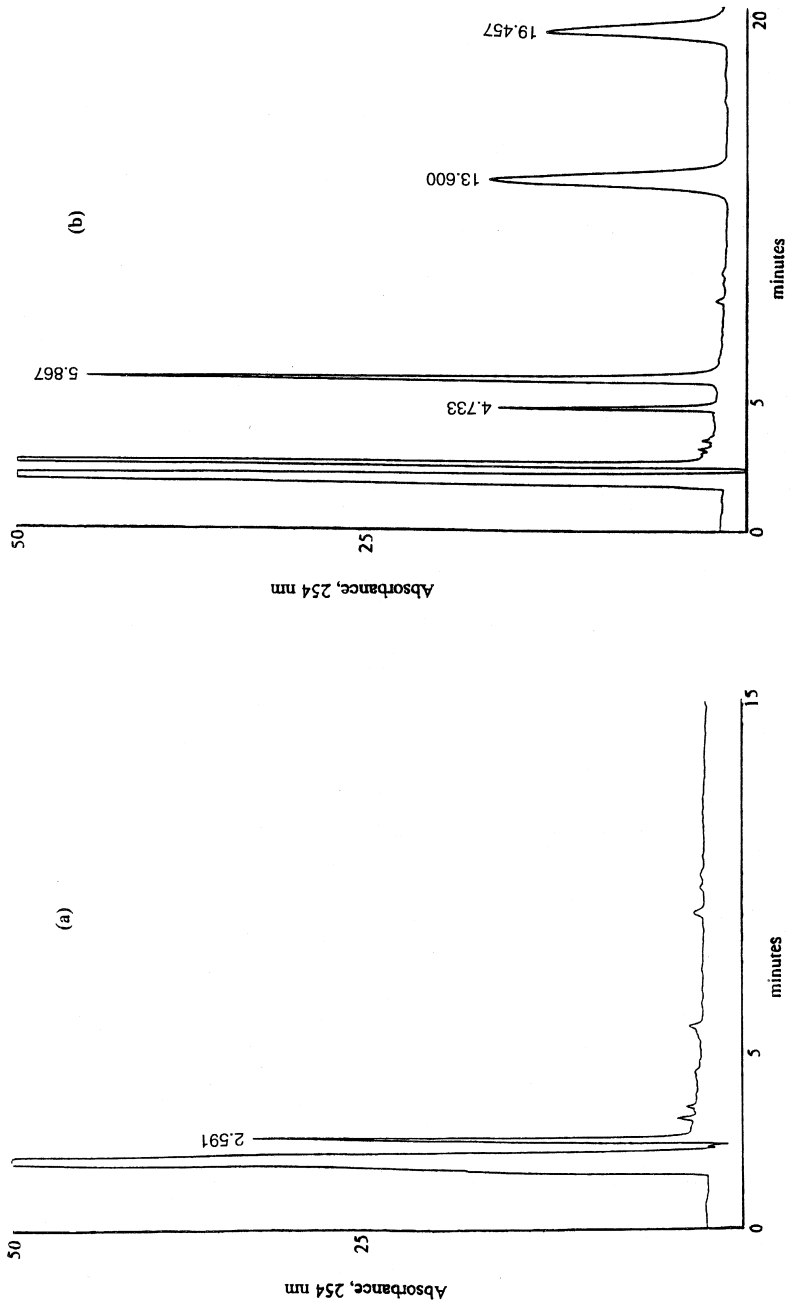

Figure 2. (a) Absorption UV spectra of 9.0 μg/mL of paracetamol (——), 3.0 μg/mL of hyoscine n-butylbromide (- - - -) and 0.3 μg/mL of lidocaine hydrochloride (.....) in water and (b) a series of absorption UV spectra of concentrations of paracetamol varied from 3.0 to 18.0 μg/mL.

Table 1

Analytical Data of the Calibration Graphs for the Determination of Hyoscine n-Butylbromide and Lidocaine Hydrochloride by High Performance Liquid Chromatography

Linearity Range of HBB (μg/mL)	Linearity Range of LID (μg/mL)	Calibration Equation[a]	r[b]
30 - 105	30 - 105	$A_{HBB} = 0.258(\pm0.028)\times C_{HBB} + 0.610(\pm).091)$	0.999
30 - 105	30 - 105	$A_{LID} = 0.260(\pm0.011)\times C_{LID} + 0.458(\pm0.084)$	0.9993
30 - 105	30 - 105	[c]$A_{HBB} = 0.237(\pm0.061)\times C_{HBB} + 0.761(\pm0.397)$	0.9994
30 - 105	30 - 105	[c]$A_{LID} = 0.231\ (\pm0.072)\times C_{LID} + 0.54(\pm0.207)$	0.9998

[a] Peak area amplitude, A, versus concentration of each compound, C, in μg/mL; six standards.
[b] Correlation coefficient.
[c] Samples were subjected to the solid phase extraction procedure prior to the HPLC analysis.

Table 2

Precision and Accuracy for the Determination of Hyoscine n-Butylbromide and Lidocaine Hydrochloride by High Performance Liquid Chromatography

Nominal Concentration (μg/mL)		Assayed Concentration of Hyoscine n-Butylbromide (μg/mL)			Assayed Concentration of Lidocaine Hydrochloride (μg/mL)		
HBB	LID	Mean±SD[c]	RSD%[a]	E_r(%)[b]	Mean±SD[c]	RSD%[a]	E_r(%)[b]
60	60	60.38±0.52	0.86	0.63	60.10±0.37	0.61	0.17
75	75	74.82±0.71	0.95	-0.24	75.30±0.38	0.50	0.40
90	90	90.30±0.67	0.74	0.33	89.95±0.58	0.64	-0.06

[a] Relative standard deviation.
[b] Relative percentage error.
[c] n = 8.

Table 3

Analytical Data of the Calibration Graphs for the Determination of Paracetamol by UV-Visible Spectrophotometry

Linearity Range of Paracetamol (μg/mL)	Calibration Equation[a]	r[b]
3 - 18	[c]$A_{242.7} = 0.665(\pm0.015)\times C_{PAR} + 0.29(\pm0.150)$	0.9994
3 - 18	[d]$A_{242.7} = 0.666(\pm0.010)\times C_{PAR} + 0.216(\pm0.104)$	0.9994

[a] Absorbance value at 242.7 nm, $A_{242.7}$, versus concentration of paracetamol, C_{PAR} in μg/mL; six standards.
[b] Correlation coefficient.
[c] Standard aqueous solutions containing paracetamol alone.
[d] Mixed standard aqueous solutions containing paracetamol, hyoscine n-butylbromide and lidocaine hydrochloride, in a ration 30:1:1.

Data for the variation of precision and accuracy given in Table 2, indicate a relative standard deviation, % RSD = 0.50 to 0.95 and a relative percentage error E_r = -0.24 to 0.63 % for the studied compounds. The statistical evaluation of the HPLC method revealed its good linearity and reproducibility and led us to the conclusion that it could have been used for the reliable determination of HBB and LID in injection forms.

The limit of detection attained as defined by IUPAC[23] $DL_{(k=3)} = k \times S_b / b$ (where b is the slope of the calibration graph and S_b is the standard deviation of the blank signal) was found to be 1.06 and 0.96 μg/mL for HBB and LID, respectively.

Evaluation of the Spectrophotometric Method

Linear relationship between the selected absorbance value at 242.7 nm, $A_{242.7}$, and paracetamol concentration was observed, results are presented in Table 3. The slope and intercept of the regression equation obtained from the analysis of mixed aqueous solutions of paracetamol, HBB and LID do not differ significantly from those obtained from the analysis of aqueous solutions of paracetamol alone. Therefore, it can be deduced that the absorbance value of the mixture, measured at 242.7 nm, is proportional to the concentration of paracetamol.

Table 4

Precision and Accuracy for the Determination of Paracetamol by UV-Visible Spectrophotometry

Nominal Concentration (μg/mL)		Assayed Concentration of Paracetamol (μg/mL)		
Paracetamol	HBB:LID in Ratio 1:1	Mean ± SD (n=8)	RSD %[a]	E_r(%)[b]
3	0.1	3.02±0.01	0.33	0.66
9	0.3	12.02±0.03	0.25	0.16
18	0.06	17.96±0.14	0.78	-0.22

[a] Relative standard deviation.
[b] Relative percentage error.

Table 5

Determination of Hyoscine n-Butylbromide, Lidocaine Hydrochloride, and Paracetamol in Laboratory Synthetic Mixtures and Commercial Injection Forms

	Recovery (Mean ± SD)%[a]		
Sample	Paracetamol	Hyoscine n-Butylbromide	Lidocaine Hydrochloride
Synthetic mixtures	100.1 ± 0.25	99.86 ± 0.25	99.37 ± 0.37
Commercial injection forms[b]	99.93 ± 0.56	99.05 ± 0.53	99.22 ± .76

[a] Mean and standard deviation for ten determinations; percentage recovery from the label claim amount.
[b] Spasmoapotel injection forms.

The precision of the spectrophotometric assay expressed as the relative standard deviation of the mean value found, and accuracy in terms of relative percentage error are reported in Table 4. The limit of detection attained as defined by IUPAC[22] was found to be 0.67 μg/mL for the UV-visible spectrophotometric determination of paracetamol.

Assay of Commercial Injection Forms

The proposed methods are suitable for the content uniformity test, where a great number of assays on individual injection forms is required. Commercially available injection forms containing mixtures of paracetamol, HBB, and LID were analyzed using the proposed methods (HPLC and UV-visible spectrophotometry).

Recovery studies were also performed by analyzing synthetic mixtures that reproduced the composition of the commercial injection forms. Recoveries achieved were in accordance with the actual content of paracetamol, HBB, and LID in the commercial injection forms. The results obtained are presented in Table 5.

In conclusion, the proposed high performance liquid chromatographic and UV-visible spectrophotometric method are specific, accurate, and precise for the analysis of hyoscine n-butylbromide, lidocaine hydrochloride, and paracetamol in injection forms. A relatively simple separation step using solid phase extraction procedure is required for the isolation of hyoscine n-butylbromide and lidocaine hydrochloride from paracetamol. These methods were applied successfully to the analysis of commercial injection forms containing these three substances.

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