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Simultaneous determination of codeine and ethyl morphine HCL in tablet formulations using LC

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Abstract

A reverse phase high performance liquid chromatography (HPLC) method was developed for the simultaneous determination of codeine (methyl morphine) and dionin (ethyl morphine hydrochloride) in antitussive analgesic tablet formulations. A C_{18} column and methanol–water (1:2) mixture mobile phase (pH 3.0) were used. Spectrophotometric detection was carried out at 210 nm. The total elution time was shorter than 7 min. This method was found to be quite precise and reproducible. This proposed method was successfully applied to the determination of codeine and ethyl morphine hydrochloride in tablets produced by the Turkish Army Drug Factory.

Keywords: Simultaneous determination; Codeine; Ethyl morphine hydrochloride; Tablet formulations; Liquid chromatography

1. Introduction

Codeine (methyl morphine, morphine monomethyl ether, morphine 3-methyl ether) has analgesic [1] and antitussive [2] effects and is widely used in cough remedy formulations [3]. Codeine phosphate is an antitussive and analgesic drug available worldwide.

A tablet formulation, called 'KO-Dİ tablet' was developed and produced by the Turkish Army Drug Factory. Each tablet contains 10 mg of codeine and 10 mg of ethyl morphine hydrochloride. No similar formulation was found in the USP XXIII (American Pharmacopoeia), BP 93 (British Pharmacopoeia) and the Turkish market. Therefore, there is no simultaneous, accurate and sensitive assay method available for both of them.

Many formulations for cough contain codeine and ethyl morphine hydrochloride but few of them contain codeine and ethyl morphine hydrochloride together in the same quantity. Some of the authors reported the results of analysis of codeine and ethyl morphine hydrochloride in tablet formulations in the literature. Bergişadi and Özsoy determined the amount of codeine and ethyl morphine hydrochloride in codeine–ethyl morphine hydrochloride combined tablets using thin layer chromatography (TLC) in 1987 [1]. Onur and Bölükbaşı have analyzed codeine and

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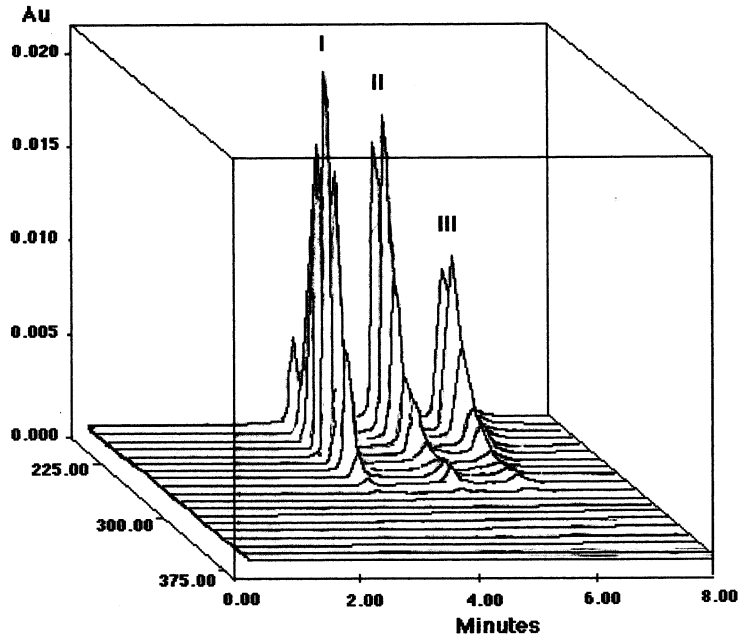

Fig. 1. A typical HPLC chromatogram of a standard solution containing ascorbic acid (I), codeine (II) and ethyl morphine hydrochloride (III). Eluting solvent, pH:3.0, methanol–water (1:2, v/v), flow rate 1.0 ml/min; ambient temperature; UV:210 nm.

Table 1
Results of the calibration curve regression analyses

Compound	Concentration (μg/ml)	A_D/A_{IS}[a] ± S.D.	Slope ± S.D.	Intercept ± S.D.	r ± S.D.
Codeine	1.0	0.8666 ± 0.002	0.6953 ± 0.0842	0.2663 ± 0.0811	0.9978 ± 0.0015
	3.0	2.3733 ± 0.005			
	4.0	3.0203 ± 0.002			
	5.0	3.5641 ± 0.002			
Ethyl morphine hydrochloride	1.0	0.5478 ± 0.003	0.4221 ± 0.0216	0.1773 ± 0.0578	0.9995 ± 0.0001
	2.0	0.9299 ± 0.005			
	3.0	1.3812 ± 0.004			
	4.0	1.7960 ± 0.004			
	5.0	2.1626 ± 0.006			

[a] Data represents ten replicate injections of standard solutions. A_D/A_{IS} is the ratio of the integrated area of the drug peak at a given concentration divided by The integrated area of internal standard (ascorbic acid) peak at a concentration of 8 μg/ml.

Table 2
Recovery of codeine and ethyl morphine hydrochloride from laboratory-made mixtures ($n = 5$)

Compound	Prepared concentration (μg/ml)	Concentration found (μg/ml)	Recovery ± S.D. (%)
Codeine	3	3.00	100.00 ± 0.42
Ethyl morphine hydrochloride	3	2.98	99.33 ± 0.60

Table 3
Intra-day precision data (repeatability) for HPLC analysis of codeine and ethyl morphine hydrochloride ($n = 5$)

Compound	Working concentration (μg/ml)	Amount found (%) (R.S.D.)	Mean ± R.S.D. (%)
Codeine	1	99.24	99.40 ± 0.37
		99.08	
		99.09	
		99.70	
		99.88	
	5	99.70	99.60 ± 0.37
		99.50	
		99.10	
		100.20	
		99.22	
Ethyl morphine hydrochloride	1	99.24	99.40 ± 0.14
		99.30	
		99.60	
		99.40	
		99.44	
	5	99.78	99.66 ± 0.30
		99.42	
		99.32	
		100.08	
		99.70	

Table 4
Summary of inter-day precision data (reproducibility) for HPLC assay of codeine and ethyl morphine hydrochloride ($n = 5$)

Compound	Concentration (μg/ml)	Amount found ± R.S.D. (%)			Mean ± R.S.D. (%) (Inter day)
		Day 1	Day 2	Day 3	
Codeine	1	99.70 ± 0.30	99.42 ± 0.30	99.70 ± 0.28	99.62 ± 0.30
	5	99.58 ± 0.52	100.1 ± 0.80	99.64 ± 0.54	99.82 ± 0.68
Ethyl morphine hydrochloride	1	99.88 ± 0.20	99.12 ± 0.24	99.30 ± 0.10	99.48 ± 0.22
	5	100.2 ± 0.40	100.3 ± 0.36	100.2 ± 0.30	100.3 ± 0.08

Table 5
Results from quantitative analysis of codeine and ethyl morphine hydrochloride in tablet formulation ($n = 5$)

Compound label	mg/tablet	Amount found (% ± R.S.D.)	Amount found (% ± R.S.D.) [4]
Codeine	10	100.33 ± 0.20	100.00 ± 1.236–100.95 ± 1.876
Ethyl morphine hydrochloride	10	99.70 ± 0.06	99.84 ± 1.720–100.88 ± 1.049

ethyl morphine hydrochloride in tablet formulations using high pressure liquid chromatography (HPLC) in 1985 [4]. They have used phenylephrine hydrochloride as an internal standard and a Bondapak CN column was chosen and water containing 2.5% glacial acetic acid (pH 2.55) has been used as a mobile phase for the analysis. They have analyzed codeine and ethyl morphine hydro-

chloride at 254 nm but in our experiments their maximum absorbance were found at 210 nm, therefore, their method was not found to be sensitive enough. There is no other method available in the literature for the simultaneous analysis of codeine and ethyl morphine hydrochloride using HPLC, therefore, it was aimed to develop a sensitive, accurate and reproducible assay method for the analysis of codeine and ethyl morphine hydrochloride using HPLC. It may also be useful for the scientists and investigators who want to analyze ascorbic acid, codeine or ethyl morphine; one of them can also be used as an internal standard for other compound analysis if a similar mobile phase and column is going to be used.

2. Materials and methods

2.1. Chromatographic conditions

A solvent delivery pump (Waters 510 HPLC isocratic pump), an automatic sample injection system (Waters 717 Plus autosampler), a 300 × 3.9 mm (C_{18}) stainless steel reverse phase column packed with 10 μm dimethyloctadecylsilan

Table 6
Stability test results of KO-Dİ tablets at 25°C

Temperature and relative humidity	Properties	22-10-1997	22-11-1997	22-12-1997	22-01-1998
25 ± 2°C, 75 ± 5%	Appearance	White	White	White	White
	Weight deviation (%)	±1.8	±1.9	±1.95	±2
	Disintegration (min)	4	4	4	4
	Codeine (%)	99.8 ± 0.1	99.8 ± 0.2	99.6 ± 0.2	99.5 ± 0.1
	Ethyl morphine hydrochloride (%)	99.6 ± 0.1	99.6 ± 0.3	99.4 ± 0.2	99.2 ± 0.2
	Humidity level of the tablet (%)	1.1	1.1	1.1	1.1
	Dissolution of the compound after 30 min				
	Codeine (%)	90.0 ± 0.2	89.6 ± 0.1	89.0 ± 0.2	88.6 ± 0.1
	Ethyl morphine hydrochloride (%)	89.0 ± 0.1	88.5 ± 0.3	88.4 ± 0.2	88.4 ± 0.1

Table 7
Stability test results of KO-Dİ tablets at 37°C

Temperature and relative humidity	Properties	22-10-1997	22-11-1997	22-12-1997	22-01-1998
37 ± 2°C, 75 ± 5%	Appearance	White	White	White	White
	Weight deviation (%)	±1.9	±1.9	±2	±2.1
	Disintegration (min)	4	5	5	7
	Codeine (%)	99.5 ± 0.1	99.3 ± 0.1	99.6 ± 0.1	99.6 ± 0.1
	Ethyl morphine hydrochloride (%)	99.6 ± 0.1	99.3 ± 0.1	99.2 ± 0.1	99.0 ± 0.1
	Humidity level of the tablet (%)	1.82	1.14	1.23	1.19
	Dissolution of the compound after 30 min				
	Codeine (%)	88.0 ± 0.2	88.6 ± 0.2	89.5 ± 0.1	88.5 ± 0.2
	Ethyl morphine hydrochloride (%)	89.1 ± 0.1	87.5 ± 0.3	87.3 ± 0.2	87.4 ± 0.3

Table 8
Stability test results of KO-Dİ tablets at 45°C

Temperature and relative humidity	Properties	22-10-1997	22-11-1997	22-12-1997	22-01-1997
45 ± 2°C, 75 ± 5%	Appearance	White	White	White	White
	Weight deviation (%)	±1.38	±1.44	±1.95	±2.2
	Disintegration (min)	7	6.5	4	5
	Codeine (%)	98.8 ± 0.1	98.8 ± 0.1	98.7 ± 0.1	98.5 ± 0.1
	Ethyl morphine hydrochloride (%)	98.5 ± 0.1	97.3 ± 0.1	99.6 ± 0.1	98.4 ± 0.1
	Humidity level of the tablet (%)	1.82	1.14	1.23	1.19
	Dissolution of the compound after 30 min				
	Codeine (%)	88.2 ± 0.2	85.7 ± 0.2	88.0 ± 0.1	87.6 ± 0.2
	Ethyl morphine hydrochloride (%)	89.2 ± 0.1	87.5 ± 0.1	88.6 ± 0.2	88.8 ± 0.2

bonded amorphous silica (Bondapak Waters, Milford, MA), and a photodiode array detector (Waters 996 photodiode array detector) were used for the HPLC analysis. The mobile phase was a mixture of methanol–water (1:2 (v/v), pH 3.0, adjusted with 10% orthophosphoric acid). The mobile phase was filtered through a 0.45 μm pore nylon membrane filter (Millipore, Bedford, MA) and degassed by sonication. The flow rate was 1.0 ml/min, and chromatogram was monitored at 210 nm.

Reagents codeine (06301079) and ethyl morphine hydrochloride (91821178) were received from Medipac, Hungary. Ascorbic acid was obtained from Sigma. Orthophosphoric acid and HPLC grade methanol were purchased from Merck. Water was demineralised and bidistilled using an Aqua Nova distillation apparatus (Degerfors, Sweden).

2.2. Standard solutions

Standard solution was prepared with the concentration of codeine 3 μg/ml, ethyl morphine hydrochloride 3 μg/ml and ascorbic acid (internal standard) 8 μg/ml in the mobile phase.

2.3. Calibration curve

Each standard solution (10 μl) was repeatedly injected into the column. Five replicates of each concentration were subjected to regression analysis and the slope and intercept values were calculated.

2.4. Pharmaceutical formulation

Each KO-Dİ tablet (Army Drug Factory, Turkey) contains 10 mg codeine and 10 mg ethyl morphine hydrochloride. The tablets were produced using wet granulation and the exact composition of the tablets was as follows:

2.4.1. Inner phase

Codeine, 10 mg; ethyl morphine hydrochloride, 10 mg; corn starch, 110 mg; lactose, 60 mg; and jelatine (5%), 2 mg.

2.4.2. Outer phase

Corn starch, 6 mg; magnesium stearate, 2 mg.

2.5. Assay

The average weight per tablet was calculated from the weight of 20 tablets. Codeine (10 mg) and 10 mg of ethyl morphine hydrochloride were accurately weighted into a 100 ml volumetric flask and dissolved in water. The solution was sonicated for 1 min and filled up to the volume with water. Then 0.1 ml of this solution was transferred to a 25 ml volumetric flask, 1 ml of internal standard solution was added and the contents were diluted to the volume with the mobile phase.

The solution (10 μl) was injected into the HPLC as mentioned above. The contents of codeine and ethyl morphine hydrochloride were calculated from the linear regression equation of the calibration curve.

2.6. Stability tests of tablets

The tablets were kept in an acclimatized chamber for four months. Different temperature levels (25, 37 and 45°C) and a certain humidity level (75.5%) were used. The tablets were then analysed to get information about compounds stability.

3. Results

The retention times for ascorbic acid (internal standard), codeine and ethyl morphine hydrochloride were observed at 3.5, 4.9 and 6.1 min, respectively. Total time of analysis was less than 7 min. Ascorbic acid was chosen as internal standard because there was no interference observed in the codeine and ethyl morphine hydrochloride region of the chromatogram. Fig. 1 shows a typical chromatogram of the standard solution.

Table 1 shows the results of the calibration curve regression analysis for codeine and ethyl morphine hydrochloride.

A mixture containing known amounts of codeine and ethyl morphine hydrochloride was used for the determination of the recovery calculations. The average percentage recoveries were found as 100.00 ± 4.69 and 99.1 ± 0.74 for codeine and ethyl morphine hydrochloride each at a concentration 3 μg/ml, respectively (Table 2).

The precision (within-day variations of replicate determinations) and the reproducibility (day to day variation of the determinations) of the proposed method are shown in Tables 3 and 4. The concentration of each compound was calculated from its peak height or peak area ratio using the regression equation. The R.S.D.s for the intra-day variations were obtained as 0.37 and 0.37% at concentrations of 1 and 5 μg/ml for codeine, and 0.14 and 034% for ethyl morphine hydrochloride at concentrations of 1 and 5 μg/ml. The R.S.D.s for inter-day precision were found as 0.30 and 0.68 for codeine and 0.08–0.22 for ethyl morphine hydrochloride at the same concentrations.

The proposed HPLC method was applied to the quantitative analysis of compounds in tablets. Table 5 summarizes the quantitative analysis results of KO-Dİ tablets.

The stability test was also performed for 4 months. Tables 6–8 summarizes the results.

4. Discussion

In order to achieve a simultaneous analysis of codeine and ethyl morphine hydrochloride under isocratic conditions, the mobile phase composition was optimized first. For instance an HPLC method has been published for the analysis of codeine and ethyl morphine hydrochloride [4] in the literature, but the shape of the peaks obtained were not symmetrical which indicated that the mobile phase and column were not suitable enough and the composition of the mobile phase needed optimization. A satisfactory separation was obtained with the mobile phase consisting of methanol–water (1:2, v/v). It was noticed that the pH of the mobile phase was also important for the resolution of the peaks. The optimum resolution of the components was obtained at pH 3.0. A reversed phase column (C_{18}) was used and the flow rate was set to 1.0 ml/min. The maximum absorption of codeine was found to be at 210 nm, therefore, this wavelength was chosen for the analysis. The retention times for ascorbic acid (internal standard), codeine and ethyl morphine hydrochloride were observed at 3.5, 4.9 and 6.1 min, respectively. Total time of analysis was less than 7 min. Ascorbic acid was chosen as internal standard because there was no interference observed in the codeine and ethyl morphine hydrochloride region of the chromatogram (Fig. 1).

The proposed method was found to be linear and reproducible. The evaluation of some validation parameters of the method as follows:

- Linearity: linearity was determined with five different levels using ten replicates. The r^2 values of the calibration curves were used. In all cases it was found to be higher than 0.995.
- The limit of detection (LOD) was found to be 0.020 and 0.035 μg/ml and the limit of quantification (LOQ) was found to be 0.100 and 0.150

μg/ml for codeine and ethyl morphine hydrochloride, respectively (*k* values were calculated as about 2.75 and 13 for LOD and LOQ).

- Precision: samples injected to the column ten times at different level and in all cases R.S.D.s were found to be less than 2%.
- Accuracy: laboratory blend was prepared using codeine and ethyl morphine hydrochloride and recovery of the active ingredients were calculated and in all cases it was in the range 96–104%.
- Selectivity/Specificity: when placebo (water) solution was analysed there was no peak observed. There were no potential interferences observed with the other chemicals and solvents. Tablets were prepared without active ingredient addition and analyzed. There were no peaks observed at the retention zone of internal standard, codeine and ethyl morphine hydrochloride.
- Stability: codeine and ethyl morphine hydrochloride dissolved in the mobile phase and kept at room temperature for 6 h and then analyzed. There were no extra peaks observed.
- Robustness: the same analysis was repeated using a different column. When another new column was used similar retention times were obtained.. The retention times for ascorbic acid (internal standard), codeine and ethyl morphine hydrochloride were observed at 3.7, 5.0 and 6.4 min, respectively.
- Ruggedness: in day and inter day analyses were performed and in all cases recovery values had less than 2% variability.
- The stability tests of the tablets were also performed and the results showed that this method can also be used in the stability tests.

5. Conclusion

The aim of the study was to develop a suitable method for the simultaneous quantitative determination of codeine and ethyl morphine hydrochloride in tablet dosage forms. The method developed allows the quantitative analysis of both compounds in tablets using the same dilution and the same injection. This is an advantage over the current USP procedure which involves a separate quantitative analysis of codeine and ethyl morphine hydrochloride using gas chromatography.

In conclusion, our results indicate that the method developed can be used for the simultaneous quantitative analysis of codeine and ethyl morphine hydrochloride in pharmaceutical formulations.

References

[1] F.T. Delbeke, M. Debackere, J. Pharm. Biomed. Anal. 11 (1993) 339–343.
[2] C.P.W.G.M. Verwey Van-Wissen, P.M. Koopman-Kimenai, J. Chromatogr. 570 (1991) 309–320.
[3] N. Bergişadi, Y. Özsoy, Acta Pharm. Turc. 29 (1987) 81–83.
[4] E. Onur, N. Bölükbaşı, STF Pharma 2 (1985) 128–131.