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TLC-UV densitometric and GC-MSD methods for simultaneous quantification of morphine and codeine in poppy capsules

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Abstract

Thin-layer chromatographic (TLC)-UV densitometric and gas-chromatographic-mass spectrometric detection (GC-MSD) methods were developed for simultaneous quantification of morphine and codeine in poppy capsules (*Papaver somniferum*). Morphine and codeine were isolated by extraction with chloroform: isopropanol (3:1, v/v) at pH = 8.5 and by solid-phase extraction on Snap-Cap cartridges at pH = 8.5. The TLC-UV densitometric quantification was performed by external standard method on silica gel plates using ethyl acetate: toluene: methanol: ammonia (68:17:10:5, v/v) as developing solvent and UV detection at 275 nm. For the GC-MSD analysis, the drugs were derivatized with acetic anhydride: pyridine (1:1, v/v) and separated on a 30 m HP5 capillary column. The quantification was performed using nalorphine as internal standard.

Keywords: Morphine; Codeine; Poppy capsules; Thin-layer chromatography-UV densitometry; Gas-chromatography-Mass spectrometry

1. Introduction

Morphine [7,8-didehydro-4,5α-epoxy-17-methylmorphinan-3,6α-diol] and codeine [7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol] are the main alkaloids in poppy seeds (*Papaver somniferum*) having pharmacological and toxicological activity. As they are widely used in therapy as well as in abuse, it is important to select performant analytical methods that allow their quick and precise quantification in biological samples as well as in pharmaceutical or illicit products.

Morphine and codeine have absorption maxima at 285 nm in aqueous acid solutions [1]. The structural differences being minimum, their simultaneous quantification is difficult when classical analytical methods are used. Modern analytical methods generally associate chromatographic and spectrometric techniques. For the analysis of opioid alkaloids in different samples, TLC and GC-MSD are frequently used [1–8].

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Table 1
Accuracy and precision of morphine calibration curves

Taken (μg/10 μl)		Found (x)	SD	RSD (%)	n	Mean accuracy (%)
2.00[a]	1.51[b]	1.49	–	–	1	−1.06
4.00	3.03	2.94	0.05	1.92	2	−2.97
5.00	3.79	3.75	0.02	0.68	3	−1.05
6.00	4.55	4.54	0.02	0.51	3	−0.22
8.00	6.07	6.09	0.02	0.38	3	+0.33
10.00	7.59	7.60	0.05	0.76	3	+0.13
12.00	9.11	9.22	0.02	0.23	3	+1.20
14.00	10.62	10.64	0.02	0.23	3	+0.18
20.00	15.18	14.90	–	–	1	−1.84

[a] Morphine hydrochloride.
[b] Morphine base.

Previous research has established the experimental conditions for the isolation and TLC separation on the main opioid alkaloids in dried mature capsules of indigenous *P. somniferum*. In this paper, two analytical methods, namely TLC-UV densitometric and GC-MSD for the simultaneous quantification of morphine and codeine in poppy capsules, are presented. The isolation of the opioid alkaloids was performed by two extraction methods: liquid–liquid extraction (L–L.E) and solid-phase extraction (SPE).

2. Materials and methods

2.1. Standards and reagents

Pharmaceutical purity standards were used [9]. Standards of morphine hydrochloride and codeine phosphate were purchased from Napofarm S.A. (Cluj-Napoca, Romania) and the internal standard (IS) nalorphine from Medimpex (Hungary).

All solvents and reagents had analytical purity. Chloroform (HPLC purity) and isopropanol (HPLC purity) were obtained from Carlo Erba (Milano, Italy) and acetic anhydride and pyridine from Merck (Darmstadt, Germany). Ethyl acetate, toluene, methanol and ammonia were purchased from Chimopat S.A. (Bucharest, Romania).

2.2. Materials and equipment

SPE was performed on Snap-Cap cartridges (300 mg C18) purchased from ABL&E (Cluj-Napoca, Romania).

TLC Alufolien Kieselgel plates (20 × 20 cm, 0.250 mm) were obtained from Merck (Darmstadt, Germany). The solutions were applied manually with a Hamilton microliter syringe (10 μl). Plates were developed in a standard chromatographic tank from Applied Science Laboratories (PA, USA). For the TLC-UV densitometry, a Desaga CD 60 densitometer was used and the GC-MSD analysis were performed with a HP 5890 II gas-chromatograph with a mass spectrometer detector HP5972, using a HP5 capillary column (30 m × 0.25 mm i.d., 0.25 μm).

2.3. Extraction procedures

2.3.1. Liquid–liquid extraction method

A precisely weighed quantity (5.003 g) of powder (sieve IV-800 μm [9]) obtained from dried mature capsules of *P. somniferum* was macerated for 3 h with 100 ml 1% acetic acid in an aqueous solution. The macerate was filtered and the filtrate, following alcalinisation with ammonia (pH = 8.5), was completed to 100 ml with distilled water. Subsequently, 10 ml from this solution was extracted with 20 ml chloroform: isopropanol

Table 2
Accuracy and precision of codeine calibration curves

Taken (μg/10 μl)		Found (x)	SD	RSD (%)	n	Mean accuracy (%)
2.00[a]	1.50[b]	1.48	–	–	1	−1.33
4.93	3.71	3.715	0.15	3.99	2	+0.14
6.00	4.52	4.52	0.08	1.69	3	−0.07
8.16	6.14	6.10	0.02	0.34	3	−0.60
10.64	8.01	8.03	0.04	0.49	3	+0.25
12.00	9.04	8.92	0.14	1.55	3	−1.33
14.00	10.54	10.65	0.14	1.32	3	+1.04
20.00	15.06	15.00	0.14	0.94	2	−0.40

[a] Codeine phosphate.
[b] Codeine base.

(3:1, v/v). The organic phase was evaporated on a water bath. The residue was dissolved in 2 ml methanol for morphine quantification and in 0.5 ml methanol for codeine quantification. Volumes of 10 μl solution per spot sample were applied on the TLC plates.

2.3.2. Solid-phase extraction method

In order to prepare the solid-phase extraction cartridge, 5 ml methanol and 5 ml distilled water were passed through the cartridge sequentially; 10 ml of filtered and alkalinised macerate were then added to the cartridge. The cartridge was washed with 5 ml distilled water and then the drugs were eluted with 2 ml methanol. The elute was collected in screw-cap tubes, evaporated to dryness and reconstituted with 2 ml methanol for morphine densitometric quantification or with 0.5 ml methanol for codeine densitometric quantification or with 2 ml acetic anhydride: pyridine (1:1, v/v) containing 250 μl nalorphine (IS) for GC-MSD analysis. In the last case, after capped and heated to 50–70°C for 1 h in a heating block, the mixture was evaporated to dryness and reconstituted with 100 μl of dry ethyl acetate; 1 μl aliquots were injected into the GC-MSD. The calibration was performed using a standard mixture that contained 300 μg morphine hydrochloride, 25 μg codeine phosphate and 250 μg nalorphine (IS) and derivatised in the same conditions as the sample.

2.3.3. Extraction recoveries

Extraction recoveries were determined analysing another standard mixture (10 mg morphine hydrochloride and 10 mg codeine phosphate in 100 ml 1% acetic acid in aqueous solution, alkalinised with ammonia to pH = 8.5), which was processed in the same conditions as the macerate for the densitometric quantification.

2.4. Chromatographic procedures

2.4.1. TLC-densitometry

The solutions were applied manually using the Hamilton microliter syringe (10 μl). TLC plates were developed at room temperature with ethyl acetate: toluene: methanol: ammonia (68:17:10:5, v/v). The distance of migration was 15 cm and the time of migration 1 h. After the plates dried, the densitometric analysis was performed at 275 nm, which corresponded to the maxima of the re-emission spectra of morphine and codeine. For the quantification of morphine and codeine, the external standard method was used.

2.4.2. GC-MSD analysis

GC-MSD quantification of morphine and codeine was performed by internal standard method. The chromatographic conditions were the following: injection port temperature: 250°C, temperature program: 140–300°C at 12°C min^{-1}, 10 min at 300°C, detector temperature: 280°C. Helium was used as carrier gas at a flow rate of 1 ml min^{-1}. The chromatogram was obtained by recording the total ion current (TIC) and the peaks were identified by comparing the mass spectra with the spectra in the Wiley 275 library.

Table 3
Precision and accuracy of 10 μg/10μl morphine hydrochloride solution control and 10 μg/10 μl codeine phosphate solution control

Parameter	Day	Taken	Found (x)	SD	RSD (%)	n	Mean accuracy (%)
Morphine base	1	7.59	7.60	0.05	0.76	7	+0.13
	2	7.59	7.62	0.03	0.42	6	+0.39
	3	7.59	7.63	0.02	0.34	6	+0.53
Overall			7.62	0.04	0.57	19	+0.39
Codeine base	1	8.01	8.03	0.04	0.49	10	+0.25
	2	8.01	8.04	0.09	1.17	9	+0.37
	3	8.01	7.98	0.05	0.65	9	−0.37
Overall			8.01	0.06	0.84	28	+0.09

3. Results and discussion

3.1. Evaluation of the densitometric method

Linear responses were obtained for both alkaloids in the concentration range 2.00–20.00 μg/10 μl. Over this concentration range, the linear regression analysis of peak areas (y) in function of concentration (x), calculated by least square method, leads to the following equations: $y = 278.2 + 143.78x$ ($r = 0.9996$, $n = 9$) for morphine and $y = 127.58 + 140.15x$ ($r = 0.9994$, $n = 8$) for codeine.

The proposed method was precise and reproducible. The mean accuracy (%) of calibration curves varied between −2.97 and +1.20% for morphine (Table 1) and between −1.33 and +1.04% for codeine (Table 2), and the values of SD were very low (< 0.15). RSD (%) of peaks areas within-day and between days were determined at a concentration of 10 μg morphine hydrochloride, respectively 10.64 μg codeine phosphate per 10 μl solution control (Table 3).

3.2. Morphine and codeine quantification in poppy capsules

3.2.1. TLC-UV densitometric analysis

The efficiency of the extraction procedures was determined by densitometry (Table 4). The results were compared by Anova test. If there was not a significant difference ($F = 1.8734$) between the two recoveries (%) of codeine, by L–L.E and SPE respectively, then the recovery for morphine is better by SPE. Anova tests showed an extremely significant difference between the results obtained by L–L.E and SPE for morphine ($F = 136.5098$).

Finally, concentrations of 223.2–232.19 and 21.04–21.98 mg of morphine and codeine base per 100 g poppy capsules were obtained, respectively (Table 5).

3.2.2. GC-MSD analysis

Morphine, codeine and nalorphine (IS) were identified by comparing the retention times (t_R) of components in the extracted poppy sample with those corresponding to morphine, codeine and nalorphine standards. Also, the mass spectra of gas-chromatographic peaks were compared with the spectra in Wiley 275 library. The superposition of spectra was 98% for morphine and 95% for codeine.

The retention indices for the three opioid alkaloids on the HP-5 capillary column in the given experimental conditions (Table 6) were computed according to the equation:

$$\mathrm{RRI} = \left(\frac{t_{RC} - t_{Rn}}{t_{Rn+1} - t_{Rn}} + n \right) \cdot 100$$

where RRI, relative retention indices; t_R, retention time; C, compound; n, alkane with n carbon atoms which eluted before the compound C; $n+1$, alkane with $n+1$ carbon atoms which eluted after the compound C.

The GC-MSD quantification of morphine and codeine in poppy capsules was performed by the internal standard method and using the following equation:

Table 4
Recovery of morphine and codeine from the standard mixture after extraction

Parameter	Liquid–liquid extraction		Solid-phase extraction	
	Morphine base	Codeine base	Morphine base	Codeine base
Taken[a]	7.59	7.53	7.59	7.53
Found (x)[b]	6.27	6.08	7.45	6.29
SD	0.24	0.34	0.06	0.16
RSD (%)	3.86	5.62	0.91	2.60
Recovery (%)	82.60	75.90	98.15	78.53

[a] μg/10 μl.
[b] $n = 6$.

Table 5
Morphine base and codeine base contents (mg %) in dried mature poppy capsules

Parameter	TLC-UV densitometry		GC-MSD
	Liquid–liquid extraction	Solid-phase extraction	
Morphine base (x)[a]	232.19	223.20	228.08
SD	19.93	7.14	5.80
RSD (%)	8.58	3.20	2.54
Codeine base (x)[a]	21.04	21.98	20.99
SD	2.05	1.38	2.23
RSD (%)	9.75	6.28	10.6

[a] $n = 6$.

$$C = \frac{W_S}{W_U} \cdot \frac{A^*_{IS}}{A^*_S} \cdot \frac{A_U}{A_{IS}} \cdot 100\%$$

where C, concentration; W, weight; A, area; IS, internal standard; S, standard; U, sample and (*) signifies the standard mixture used for calibration [10].

Table 6
GC separation parameters for morphine, codeine and nalorphine on a HP5 capillary column

Alkaloid	Retention times (t_R)	Relative retention indices (RRI)
Morphine diacetylated	14.87[a]	2714.5
Codeine acetylated	13.86	2588.25
Nalorphine diacetylated	15.95	2849.5

[a] Minimum.

The concentrations obtained were 228.08 mg % morphine and 20.99 mg % codeine, that confirm the TLC-UV densitometry results (Table 5).

Anova tests did not provide any significant differences between the results obtained by L–L.E ~ UV densitometric method, SPE ~ UV densitometric method and SPE ~ GC-MSD method respectively ($F = 0.7563$ and 0.5053 for morphine and codeine quantifications, respectively).

Literature data reported a morphine content in Romanian poppy capsules (*P. somniferum*) of 450 mg % (up a maximum concentration of 640 mg %) [11], but the alkaloid content is function of many factors such as evolution condition of vegetable, climate, geographical area, etc.

4. Conclusions

The concentrations in morphine and codeine of dried mature capsules of Romanian *P. som-*

niferum were determined using two methods: TLC-UV densitometry (external standard technique) and GC-MSD (internal standard nalorphine). The densitometric method was validated and used to determinate the efficiency of two extraction procedures: morphine and codeine isolation by liquid–liquid extraction with chloroform: isopropanol (3:1, v/v) at pH = 8.5 and by solid-phase extraction on Snap-Cap cartridges at pH = 8.5, respectively. The results obtained by the three methods: L–L.E ~ UV densitometry, SPE ~ UV densitometry and SPE ~ GC-MSD, were correlated between them and provided a high morphine content (223.2–232.19 mg %) and a low codeine content (21.04–21.98 mg %) of the Romanian poppy capsules.

TLC-UV densitometric method validated and presented in this paper can also be used in the quality control of pharmaceutical preparations of morphine and codeine.

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