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Determination of codeine and morphine in Brown Mixture (opium preparations) in Taiwan using SPE and GC/MS

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Abstract

Brown Mixture (BM), containing opium powder (10.0-10.5% morphine), opium tincture (0.9-1.1% morphine), or camphorated opium tincture (0.045-0.055% morphine), is a popular remedy, while heroin use is prohibited in Taiwan. Thus, for specimens that are tested positive for morphine, specimen donors' claims of BM use have to be adequately addressed. This study was designed to develop and validate a gas chromatography-mass spectrometry (GC-MS) method for simultaneous quantitation of codeine and morphine in BM in order to gain further insights related to the ingestion of BM. The effectivenesses of solid-phase extraction (SPE) cartridges and deuterated analogs (d_3- and d_6-) of codeine and morphine in the sample preparation and quantitative determination processes were evaluated. Conclusions were reached that codeine and morphine can be effectively analyzed, qualitatively and quantitatively, as trimethylsilyl derivatives, using selective ion monitoring of the following ions: *m*/*z* 371, 372, 343 for codeine and 377, 378, 349 for codeine-d_6; 429, 414, 401 for morphine and 435, 420, 404 for morphine-d_6. The overall protocol achieved the following results when applied to the analysis of 1 mL opiates-free BM specimens fortified with 50–1500 ng/mL codeine and morphine: recovery, 90.00–100.87%; interday and intraday precision ranges, 1.10–6.46% and 1.04–3.91%, respectively; linearity, $r^2 > 0.997$; limits of detections, 30 ng/mL for codeine and 20 ng/mL for morphine; limits of quantitation, 30 ng/mL for codeine and 40 ng/mL for morphine. Eight BM products (5 tablets and 3 solutions) from 7 different manufacturers were analyzed for their morphine and codeine contents. The contents of morphine and codeine in the tablets were found very consistent, with the morphine-to-codeine concentration ratios ranging from 8.66 to 9.09 (mean = 8.85, standard deviation = 0.16), but were found to vary considerably in the 3 BM solutions (morphine-to-codeine concentration ratios: 2.19, 2.34, 3.28).

Keywords: Brown Mixture, opium preparations, deuterated internal standards, GC-MS, forensic science

Introduction

Urine drug testings are often performed in the criminal justice system to verify whether arrestees of concern are indeed drug users. Those tested positive for morphine often claimed that the positive results were caused by the ingestion of opiates-containing medications, including Brown Mixture (BM)[+], a popular cold remedy in Taiwan. "False" positive results caused by medications do happen and human rights may be compromised under this circumstance. According to a survey, 800 prescription drugs containing opium/morphine/codeine are currently available on the market, of which the ingestion may cause positive test results.

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+ Brown Mixture is a legal prescription drug in Taiwan. Each tablet contains 2.5 mg opium powder (10.0–10.5% morphine), 0.48 mL glycyrrhiza extract, 1.0 mg antimony potassium tartrate, 2.5 mg benzoic acid, 1.5 mg camphor, 0.0015 mL anise oil, and lactose.

Brown Mixture (compound Glycyrrhiza mixture) is the most common variation of these products.

Brown Mixture is sold in the forms of tablet and solution in Taiwan. Seven different manufacturers produce a total of 8 different BM products. Among those, 5 are tablets and 3 are solutions. The tablets all contain opium powder, while the solutions contain opium tincture or camphorated opium tincture. According to the Chinese Pharmacopoeia [1], BM contains 2.5 mg of powdered opium per tablet. Powdered opium includes 10.0–10.5% anhydrous morphine [2]; there is 0.90–1.10 g anhydrous morphine in every 100 mL of opium tincture, and 45–55 mg anhydrous morphine in every 100 mL of camphorated opium tincture. The ingestion of these products could cause the detection of morphine and codeine in urine and the test result is unfortunately similar to what is derived from a heroin addict.

Abundant studies related to the detection of opiates resulting from the ingestion of opiates-containing drugs or poppy seeds have been reported. These studies examined the metabolism of codeine followng the ingestion of cough medications [3], metabolism products and their quantities [4], codeine-to-morphine ratio characteristics [5], the kinetics of codeine and morphine in the metabolism process [6]. For poppy seeds related studies, focuses were placed on the levels of codeine and morphine detected in urine following the ingestion of poppy seeds [7–9] and possible differentiation on the source of codeine and morphine found in urine [10].

Since BM is not popular elsewhere, only a few studies related to this drug have been reported in the literature. The (Taiwanese) National Bureau of Controlled Drugs published a report employing HPLC to quantify ingredients in Brown Mixture [11]. Another report by the (Taiwanese) Bureau of Food and Drug examined the levels and ratios of codeine and morphine detected in urine following the ingestion of codeine-containing cold syrup [12].

It has been well established that the detection of 6-acetylmorphine (6-AM) is a definite proof of heroin ingestion [13–15]. However, with its short half-life (0.6 hour [15]) and narrow detection window (< 8 hours), characterization of other analytes derived from the ingestion of other opiates-containing products can often faciliate the scientific investigation process. With this in mind, this study was designed to develop and validate an effective gas chromatography-mass spectrometry (GC–MS) protocol that can be conventionally applied to determine the contents of codeine and morphine in BM products available in Taiwan. Hopefully, additional insights gained through this study can be helpful to distinguishing the source of codeine and morphine detected in routitne urine drug testing practices.

Materials and methods

Chemicals and reagents

All solvents and reagents were HPLC grade and purchased from J.T. Baker Inc. (Phillipsburg, NJ, US). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Aldrich Chemical (Milwaukee, WI, US). Codeine, codeine-d_3, codeine-d_6, morphine, morphine-d_3, and morphine-d_6 solutions (1.0 mg/mL in methanol) were provided by Cerilliant Corporation (Austin, TX, US). Solid-phase extraction sorbents Isolute® HCX (130 mg) was obtained from International Sorbent Technology Ltd. (Mid Glamorgan, U.K.).

Sample preparation

For the analysis of morphine and codeine compositions in BM tablet, 100 mL deionized water was added to a 200-mL volumetric flask containing one BM tablet and mixed by sonication for 1 hour then filled to 200 mL with deionized water and thoroughly mixed. For the analysis of BM solutions, 1 mL BM solution was transferred to a 100-mL volumetric flask; then filled to 100 mL with deionized water and thoroughly mixed.

For solid-phase extraction, the manufacturers' instructions were followed. Typically, the 1 mL BM solution samples (diluted as described) with 50 μL of a 2-internal standard mixture (5 μg/mL each of codeine-d_6 and morphine-d_6), then adjusted to pH 6.0 with 1 mL 0.1M phosphate buffer. Solid-phase extraction columns were conditioned by the addition of 1 mL methanol, 1 mL deionized water, and followed by 1 mL phosphate buffer (pH 6.0). The BM solution samples were applied to the columns requiring at least 2 min to flow through. The columns were then washed with 2 mL deionized water, 2 mL 0.01N HCl, and 2 mL methanol and dried for 2 min. The analyte was eluted with 2 mL mixture of ethyl acetate, methanol, and ammonium hydroxide (73:25:2, v/v). The resulting extracts were dried under a stream of nitrogen at 50 ºC.

For derivatization, 50 μL ethyl acetate and 50 μL MSTFA were added to the residue in the screw-top tube prepared previously. The capped tube was vortex-mixed for 20 s and then heated at 100 ºC for 15 min. The reaction mixture was allowed to cool to room temperature prior to GC–MS analysis.

GC-MS analysis

An Agilent 6890N GC/5973N MSD, operated at 70 eV with ion source temperature at 230 ºC, was used in this study. The gas chromatograph was equipped with a 30-m Hewlett-Packard (Andover, MA, US) HP-1MS fused silica capillary column (0.25-mm ID; 0.25-μm film thickness). The injector and interface temperature were maintained at 260 and 280 ºC, respectively. The inlet pressure was held at 5 psi for 1 min, then programmed to 20 psi at 2 psi/min, and held for 6.5 min. Oven temperature was held at 60 ºC for 1 min, then programmed to 300 ºC at 30 ºC /min, and held at the final temperature of for 5 min. The following parameters were used for injecting samples into the GC-MS system: sample size, 1 μL; injection mode, splitless; injector purge-off duration, 1 min.

Ions selected for monitoring the resulting TMS-derivatives were *m*/*z* 371, 372 and 343 for codeine; 377, 378 and 349 for codeine-d_6; 429, 414 and 401 for morphine; and 435, 420 and 404 for morphine-d_6. The first ion listed for each compound was used for quantitation using a six-point (50, 125, 250, 500, 1000, 1500 ng/mL) calibration protocol.

Selection of ions for selective ion monitoring

Codeine, morphine and the two perspective internal standards (d_3-codeine, d_6-codeine and d_3-morphine, d_6-morphine) were derivatized and analyzed using the procedure and parameters described above. Full-scan spectra of the derivatization products were first collected and examined for preliminary selection of candidate ions for collecting SIM data for qualitative and quantitative analysis purposes. SIM data of these ions were then collected to provide more definite information on cross-contributing the intensity of ions designated for the analyte by the perspective internal standard and vice versa.

Results and discussion

Selection of deuterated internal standards and ions

Codeine. The full-scan spectra of codeine (analyte) and its two deuterated analogs (d_3-codeine and d_6-codeine) were shown in Figure 1. The mass spectrum of d_6-codeine shows 100%, 27.7%, and 17.4% relative intensities for *m*/*z* 377, 378, and 349 ions (Table 1). Since the full-scan mass spectrum of codeine all shows 0% for these three ions (Table 1). They appear to be free of interference and can be designated for d_6-codeine in SIM data acquisition. The full-scan mass spectrum of codeine shows 100%, 27.9%, and 18.0% for 371, 372, and 343, respectively (Table 1). The relative intensities of these ions observed in the full-scan mass spectrum of d_6-codeine shows 0%, 0.1% and 2.1% for these three ions (Table 1), indicating no or low cross-contribution and suitable for SIM data acquisition for codeine. The data of SIM peak area integration in Table 2 indicate that cross-contributions to *m*/*z* 371, 372, 343 (designated for codeine), 377, 378, and 349 (designated for d_6-codeine) are all below 3%. These six ions appear to be free or low of interference and suitable for quantitation analysis.

The full-scan mass spectrum and SIM peak area integration data of d_3-codeine/codeine are shown in Table 1 and Table 2. Data in Table 2 indicated that low cross-contribution to *m*/*z* 371, 372, 343 (designated for codeine); 374, 375, 346 (designated for d_3-codeine) are 0%, 2.3%, 2.9%; 1.4%, 0%, and 1.4%, respectively. Therefore, d_6-codeine is a preferable internal standard than d_3-codeine for the quantitation codeine by GC/MS.

Relative intensity and cross-contribution data are shown in Table 1 and 2 indicated that selections of *m*/*z* 371, 372, 343 (for codeine) and *m*/*z* 377, 378, 349 (for d_6-codeine) provides ions that have significant intensities (> 15%) with no cross-contribution interference. The most intense ions *m*/*z* 371 and 377 were used for quantification purposes. These ions were then used for the rest of this study.

Morphine. Ion pair selection procedures were more fully described in the codeine section. The full-scan spectra of morphine (analyte) and its two deuterated analogs (d_3-morphine and d_6-morphine) are shown in Figure 2. The mass spectrum of d_6-morphine shows 100%, 40%, and 30% relative intensities for *m*/*z* 435,

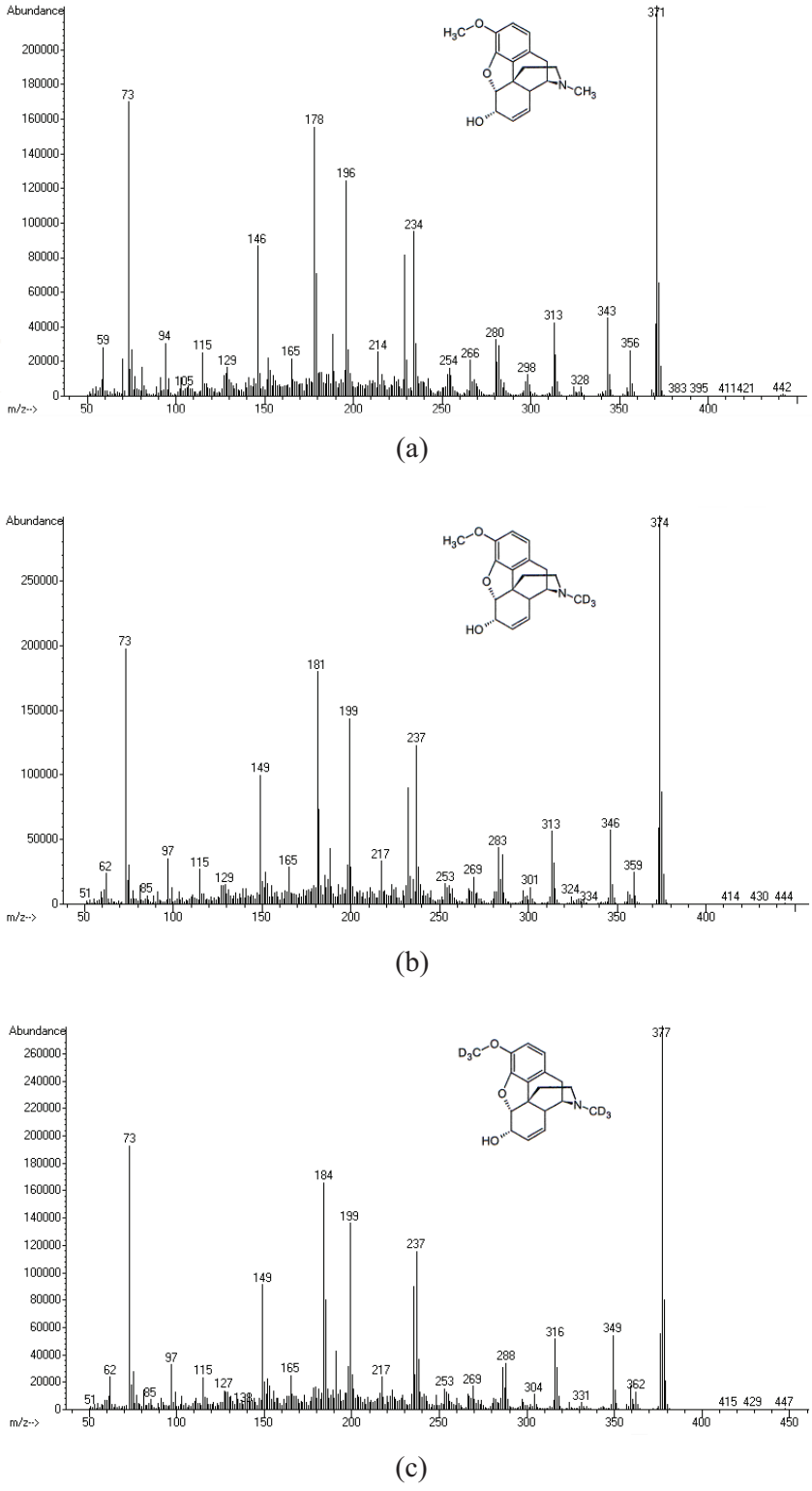

Fig.1 Full-scan mass spectra of TMS-derivatized codeine (a), d_3-codeine (b), and d_6-codeine (c).

Table 1. Relative intensity (in %) of selected ions from the full-scan mass spectra of TMS-derivatized codeine, d_3-codeine and d_6-codeine.

Ion (*m/z*)	codeine	d_3-codeine	d_6-codeine
Ion designated for codeine			
372	27.89	1.85	0.11
371	100.00	0.16	0.03
356	11.19	20.03	0.66
343	18.00	2.91	2.12
313	16.11	87.64	3.25
234	28.92	3.31	7.57
229	23.32	10.40	9.01
196	33.64	4.87	7.14
178	42.09	6.34	7.19
146	21.98	3.70	2.21
Ion designated for d_3-codeine			
375	0.40	38.06	–[a]
374	1.47	100.00	–
359	2.39	7.93	–
346	1.46	17.31	–
313	114.10	16.57	–
237	24.37	34.65	–
232	6.18	24.16	–
199	7.34	36.22	–
181	9.04	47.31	–
149	6.37	26.02	–
Ion designated for d_6-codeine			
378	0.00	–[a]	27.66
377	0.00	–	100.00
359	3.11	–	5.94
349	0.00	–	17.43
316	5.68	–	15.74
237	28.19	–	29.19
235	48.22	–	21.99
199	8.35	–	31.02
184	6.06	–	36.95
149	7.98	–	20.23

a Since d_3- and d_6-codeine will not be used together, the intensity data of these ions are irrelevant.

Table 2. SIM cross-contribution data of ion designated for the codeine and perspective internal standards.

Deuterated Analog	Analyte			Deuterated analog		
d_3-Codeine	372 (2.3%)	371 (0%)	356 (25%)	375 (0%)	374 (1.4%)	359 (0%)
	343 (2.9%)	313 (101%)	234 (3.9%)	346 (1.4%)	313 (108%)	237 (14%)
	229 (11%)	196 (5.4%)	178 (6.9%)	232 (5.8%)	199 (7.1%)	181 (9.0%)
	146 (3.8%)			149 (5.9%)		
d_6-Codeine	372 (0%)	371 (0%)	356 (0%)	378 (0%)	377 (0%)	359 (2.6%)
	343 (2.5%)	313 (3.9%)	234 (11%)	349 (0%)	316 (5.4%)	237 (13%)
	229 (12%)	196 (8.9%)	178 (9.8%)	235 (44%)	199 (7.2%)	184 (5.3%)
	146 (3.2%)			149 (6.5%)		

420, and 404 ions (Table 3). Since the full-scan mass spectrum of morphine all shows 0%, 0% and 2.7% for these three ions (Table 3). They appear to be free or low of interference and can be designated for d_6-morphine in SIM data acquisition. The full-scan mass spectrum of morphine shows 100%, 44%, and 27% for 429, 414, and

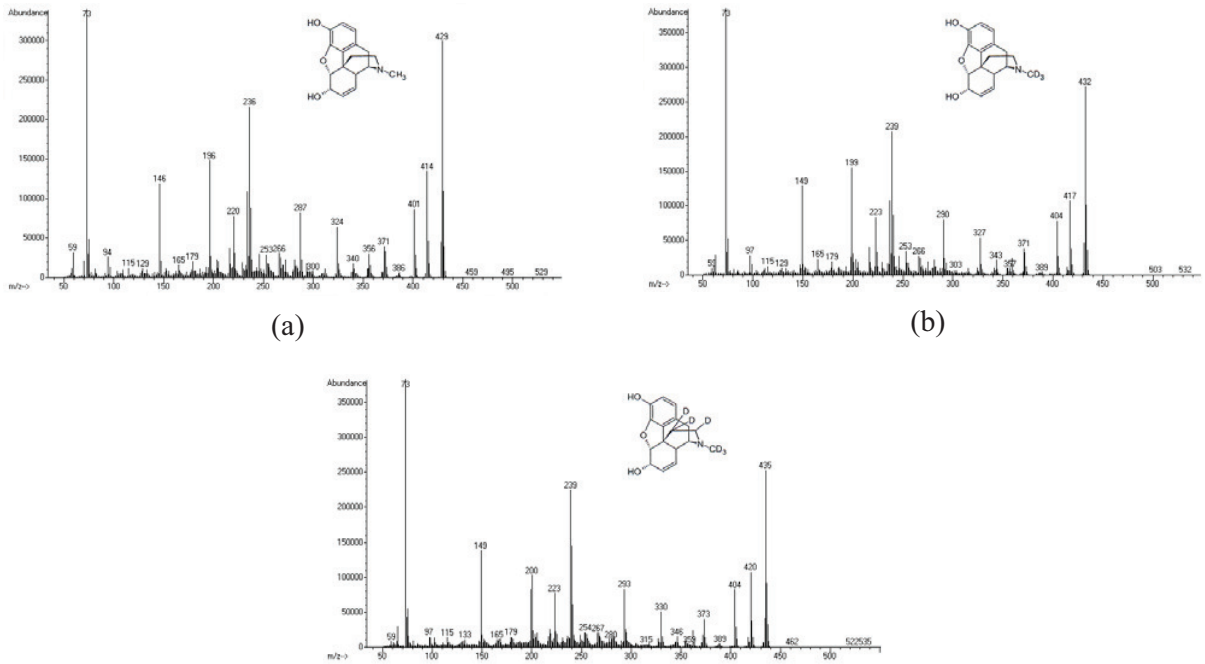

Fig.2 Full-scan mass spectra of TMS-derivatized morphine (a), d_3-morphine (b), and d_6-morphine (c).

401, respectively (Table 3). The relative intensities of these ions observed in the full-scan mass spectrum of d_6-morphine shows 0%, 0.4% and 1.3% for these three ions (Table 3), indicating no cross-contribution and suitable for SIM data acquisition for morphine. SIM peak area integration data are shown in Table 4 indicate that cross-contributions to *m/z* 429, 414, 401 (designated for morphine); 435, 420, 404 (designated for d_6-morphine) are all below 3%. These six ions appear to be free of interference and suitable for quantitation analysis.

The full-scan mass spectrum and SIM peak area integration data of d_3-morphine /morphine are shown in Table 3 and Table 4. Data shown in Table 4 indicate that high cross-contributions to *m/z* 429, 414, 401 (designated for morphine); 432, 417, 404 (designated for d_3-morphine) are 0%, 8.7%, 1.2%; 3.0%, 3.3%, and 2.7%, respectively. The internal standard d_6-morphine appears indicated no cross-contribution more suitable than d_3-

Table 3. Relative intensity (in %) of selected ions from the full-scan mass spectra of TMS-derivatized morphine, d_3-morphine and d_6-morphine.

Ion (*m/z*)	morphine	d_3-morphine	d_6-morphine
Ion designated for morphine			
430	35.67	1.39	0.14
429	100.00	0.13	0.04
414	44.28	7.58	0.35
401	27.29	0.64	1.25
324	18.66	15.22	2.44
287	22.34	9.27	3.90
236	54.22	5.96	5.70
220	19.78	4.31	9.13
196	36.59	2.24	3.93
146	30.96	0.96	1.06
Ion designated for d_3-morphine			
433	1.44	35.81	–[a]
432	2.95	100.00	–
417	3.54	37.32	–
404	2.85	26.45	–
327	6.68	15.75	–
290	2.17	22.58	–
239	4.36	52.20	–
223	11.77	20.87	–
199	4.32	38.84	–
149	3.07	32.22	–
Ion designated for d_6-morphine			
436	0.00	–[a]	35.36
435	0.00	–	100.00
420	0.00	–	40.06
404	2.68	–	30.14
330	2.24	–	14.78
293	23.22	–	23.17
239	4.51	–	54.26
223	14.54	–	18.14
199	9.31	–	19.36
149	3.51	–	30.31

[a] Since d_3- and d_6-morphine will not be used together, the intensity data of these ions are irrelevant.

Table 4. SIM cross-contribution data of ion designated for the morphine and perspective internal standards.

Deuterated Analog	Analyte			Deuterated analog		
d_3-Morphine	430 (1.8%)	429 (0%)	414 (8.7%)	433 (1.5%)	432 (3.0%)	417 (3.3%)
	401 (1.2%)	324 (17%)	287 (10%)	404 (2.7%)	327 (7.5%)	290 (2.2%)
	236 (6.6%)	220 (4.6%)	196 (2.9%)	239 (3.7%)	223 (11%)	199 (4.0%)
	146 (1.1%)		149 (2.3%)			
d_6-Morphine	430 (0%)	429 (0%)	414 (0%)	436 (0%)	435 (0%)	420 (0%)
	401 (1.5%)	324 (2.7%)	287 (4.9%)	404 (2.6%)	330 (2.1%)	293 (22%)
	236 (7.4%)	220 (11%)	196 (5.5%)	239 (3.6%)	223 (13 %)	199 (8.0%)
	146 (1.4%)		149 (2.0%)			

morphine for quantitation codeine by GC/MS.

Relative intensity and cross-contribution data shown in Table 3 and 4 indicated that selections of *m*/*z* 429, 414, 401 (for morphine) and *m*/*z* 435, 420, 404 (for d_6-morphine) provides ions that have significant intensities (> 25%) with no cross-contribution interference. The most intense ions *m*/*z* 429 and 435 were used for quantification purposes. These ions were then used for the rest of this study.

Extraction efficiency

Recovery efficiencies of the extraction procedure for the two analytes (codeine and morphine) were evaluated using two sets of standards at six concentration levels: 50, 125, 250, 500, 1000, 1500 ng/mL. For Set I, opiates-free BM solutions were spiked with the analytes and extracted without the internal standards. Internal standards (50 µL of 5 µg/mL solution) were then added to the resulting extracts and to the second set (Set II) of standards containing the same respective amounts of the analytes. Samples in both sets were then derivatized and analyzed by the GC–MS protocol. Recoveries were calculated by dividing the amounts of the analytes resulting from Set I by the amounts derived from the corresponding samples in Set II. As shown in Table 5, the ranges, means, and standard deviations of extraction recoveries were: 90.00-100.87%, 96.57%, and 3.79% for codeine; 90.58-96.77%, 93.34%, and 2.38% for morphine.

Calibration and linearity

Linearity of this analytical protocol was evaluated using a set of standards containing all analytes at the following concentration levels: 50, 125, 250, 500, 1000, 1500 ng/mL. Good linearity is demonstrated by the concentration versus peak response plots shown in Figure 3. The regression equations of the curve and their correlation coefficients were also calculated, and the $r^2 > 0.997$.

Precision and accuracy

The intra- and inter-day precisions of the analytical procedure were also evaluated at six concentration levels. Three sets of standards at these concentration levels were analyzed at the same day or in three consecutive days and the resulting data are shown in the last two columns of Table 6 with the following ranges: 1.09-6.15% for codeine; 1.04-6.46% for morphine.

For the evaluation of the assay's *limits of detection and quantitation* (LOD and LOQ), four sets of standard solutions with the following concentrations were prepared: 5, 10, 20, 30, 40, 50 ng/mL. One set was used as the calibrators, while the other three sets were used as "test specimens". All four sets were processed as one analytical batch. Limits of detection and quantitation (LOD, LOQ) were defined using the commonly adapted criteria, i.e., reasonable agreements of the retention time and ion ratio information that were derived from the standard and the test specimen in the same analytical

Table 5. Percent recovery data (mean, standard, relative standard deviation) of codeine and morphine spiked into opiates-free Brown Mixture.

Drug and concn (ng/mL)	Replicate	Mean	Std dev	Relative std dev
Codeine				
50	3	90.00	2.30	2.56
125	3	94.92	0.87	0.92
250	3	97.16	0.74	0.76
500	3	97.40	0.84	0.86
1000	3	99.09	0.50	0.50
1500	3	100.87	2.15	2.13
Morphine				
50	3	90.58	1.10	1.21
125	3	91.23	0.24	0.26
250	3	92.29	1.78	1.93
500	3	94.86	2.63	2.77
1000	3	94.33	1.21	1.28
1500	3	96.77	4.60	4.75

batch. Using these criteria, the LOQs for the protocol hereby established were 30 ng/mL for codeine and 40 ng/mL for morphine, while the LODs were 30 ng/mL for codeine and 20 ng/mL for morphine.

Morphine and codeine in Brown Mixture

Result derived from the analysis of 5 BM tablets and 3 BM solutions from different manufacturers are shown in Table 7. BM tablets containing opium powder (10.0-10.5% morphine) were found to include 244.70 to 294.76 μg of morphine and 28.25 to 32.44 μg of codeine (per tablet). The "morphine-to-codeine" ([M]/[C]) ratios in these BM tablets range from 8.66 to 9.09 with an average of 8.85 ± 0.16 (standard deviation). BM solution from manufacturer F contains opium tincture (0.9–1.1% morphine) and was found to contain 128.78 μg of morphine and 58.86 μg of codeine (per milliliter), [M]/[C] = 2.19. The other BM solution from manufacturer G and H contains camphorated opium tincture (0.045–0.055% morphine) was found to contain: 53.88 and 44.43 μg of morphine; 23.03 and 13.54 μg of codeine (per milliliter); [M]/[C] = 2.34 and 3.28, respectively. The codeine and morphine contents differed in different products, therefore the levels of codeine and morphine detected also differed.

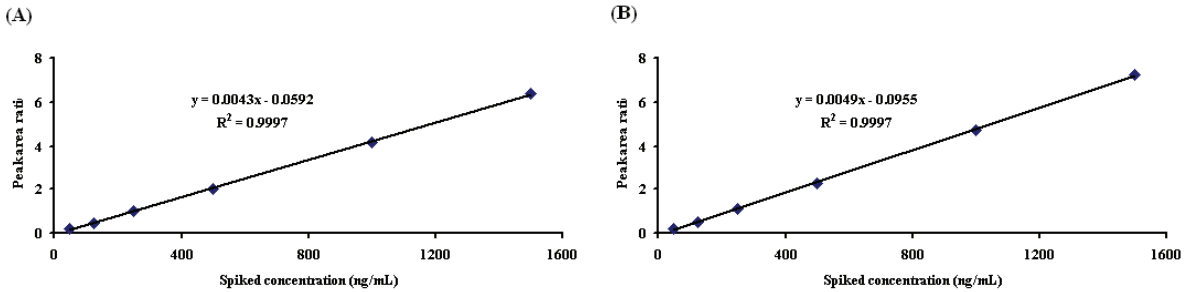

Fig.3 Calibration curves for the analysis of codeine (A) and morphine (B) from 50 to 1500 ng/mL using the d_6-codeine and d_6-morphine as internal standard.

Table 6. Intra- and inter-day precision data (mean, standard deviation, relative standard deviation) for the analysis of codeine and morphine spiked into opiates-free Brown Mixture.

Concn		Intra-day			Inter-day		
(ng/mL)	Replicate	Mean	Std dev	Rel std dev	Mean	Std dev	Rel std dev
Codeine							
50	5	50.90	0.96	1.90	51.86	0.58	1.12
125	5	122.13	1.93	1.58	128.15	2.76	2.15
250	5	249.17	6.37	2.56	252.14	6.76	2.68
500	5	505.56	5.50	1.09	501.15	12.84	2.56
1000	5	1004.57	39.25	3.91	1050.10	61.38	5.84
1500	5	1511.11	36.10	2.39	1531.64	94.19	6.15
Morphine							
50	5	50.00	1.68	3.37	52.07	0.57	1.10
125	5	125.88	1.31	1.04	120.43	3.12	2.59
250	5	248.73	2.70	1.09	253.77	11.33	4.46
500	5	512.58	17.98	3.51	491.94	31.77	6.46
1000	5	1010.83	24.00	2.37	1032.30	53.23	5.16
1500	5	1515.76	33.41	2.20	1502.40	69.21	4.61

Table 7. Quantitation of codeine and morphine (in μg/tab or μg/ml) in BM from different manufacturers.

			Mean ± S.D.		[M]/[C]
Manufacturer*	Dosage form	n	Morphine	Codeine	Ratio
A	Tablet	5	265.06 ± 2.61	29.83 ± 0.27	8.88
B	Tablet	5	244.70 ± 2.95	28.25 ± 0.50	8.66
C	Tablet	5	260.41 ± 15.85	29.63 ± 1.59	8.79
D	Tablet	5	263.09 ± 4.71	29.86 ± 0.50	8.81
E	Tablet	5	294.76 ± 8.16	32.44 ± 1.18	9.09
F	Solution	5	128.78 ± 1.07	58.86 ± 0.48	2.19
G	Solution	5	53.88 ± 1.63	23.03 ± 0.36	2.34
H	Solution	5	44.43 ± 0.84	13.54 ± 0.19	3.28

* The brand names and manufacturers of these products are: A, Compound Glycyrrhizae Tablets (Curie Taiwan Biotech Co. Ltd.: Taoyuan Hsien, Taiwan); B, Opium and Glycyrrhiza Mixture Tablets (Astar: Hsin-Chu Hsien, Taiwan); C, Brown Mixture Opium Tablets (Washington Pharmaceutical Co. Ltd.: Kaohsiung Hsien, Taiwan); D, Brown Mixture Tablet (with Opium) (Center Laboratories, Inc.: Hsin-Chu Hsien, Taiwan); E, Brown Mixture Tablets (Johnson Chemical Pharmaceutical Works, Ltd.: Taipei Hsien, Taiwan); F, Brown Mixture Liq. (with Opium) (Health Chemical & Pharmaceutical, Co. Ltd.: Taichung Hsien, Taiwan); G, Liquid Brown Mixture (with Opium) (Center Laboratories, Inc.: Hsin-Chu Hsien, Taiwan); H, Compound Glycyrrhiza Mixture (Synpac-Kingdom Pharnaceutical Co. Ltd.: Taipei Hsien, Taiwan)

Conclusions

In this study, we have established a GC-MS method for the determination of codeine and morphine in BM. Solid phase extraction cartridges and deuterated analogs (d_3- and d_6-) of the analytes were evaluated and conclusions were reached that codeine and morphine in BM can be effectively analyzed, qualitatively and quantitatively. The sensitivity and analytical time of the method are more favorable than those derived from the corresponding HPLC-based approach.

References

1. *Chinese Pharmacopeia*, 2th ed. Ministry of Interior, Executive Yuan, Taiwan, 1959, pp 326-327.
2. Lee ML. *Chinese Pharmacopeia*, 5th ed. Department of Health, Executive Yuan, Taiwan, 2000, pp 903–906.
3. Posey BL, Kimble SN. High performance liquid chromatographic study of codeine, narcodeine, and morphine as indicators of codeine ingestion. J Anal Toxicol 1984;8: 68-74.
4. Soloman MD. A study of codeine metabolism. Clin Toxicol 1974;7:255-257.
5. Datt MC, Lo DS T, Ng DLK, Woo SO. Gas chromatographic study of the urinary codeine-to-morphine ratios in controlled codeine consumption and in mass screening for opiates drugs. J Chromatogr 1983;267:117-124.
6. Cone EJ, Welch P, Paul BD, Mitchell JM. Forensic drug testing for opiates, III. Urinary excretion rates of morphine and codeine following codeine administration. J Anal Toxicol 1991;15:161-166.
7. Struempler RE. Excretion of codeine and morphine following ingestion of poppy seeds. J Anal Toxicol 1987;11: 97-99.
8. Zebelman AM, Troyer BL, Randall GL, Batjer JD. Detection of morphine and codeine following consumption of poppy seeds. J Anal Toxicol 1987;11: 131-132.
9. Fritschi G, Prescott Jr, WR. Morphine levels in urine subsequent to poppy seed consumption. Fornsic Sci Int 1985;27:111-117.
10. ElSohly MA, Jones AB. Morphine and Codeine in biological fluids: approches to source differentiation. Forensic Sci Rev 1989;1:13-22.
11. Chang S-G, Wang C, Chen J-J, Chin F-S, Li J-H. HPLC analysis of morphine in tablte mixture glycyrrhizin composite. J Food Drug Anal 1996;4:41-47. (Taiwan)
12. Chang B-L, Huang M-K. Urinary excretion of codeine and morphine following the administration of codeine-containing cold syrup. J Anal Toxicol 2000;24:133-139.
13. Paul BD, Mitchee JM, Mell Jr, LD, Irving J. Gas chromatography/electron impact mass fragmentometric determination of urinary 6-acetylmorphine, a metabolite of heroin. J Anal Toxicol 1989;13:2-7.
14. Feln J, Megges G. Detection of O^6-monoacetyl morphine in urine samples by GC/MS as evidence heroin use. J Anal Toxicol 1985; 9:134-138.
15. Cone EJ, Welch P, Mitchell JM, Paul BD. Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. J Anal Toxicol 1991; 15:1-7.