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LC and LC-MS-MS analyses of undeclared codeine in antiasthmatic Chinese proprietary medicine

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Abstract

This paper describes an accurate and sensitive reversed phase high-performance liquid chromatographic (RP-HPLC) method for the detection and quantification of undeclared codeine in a Chinese Proprietary Medicine (CPM) for asthma. A rapid and specific liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) method was applied to confirm the presence of codeine by selected reaction monitoring (SRM). Codeine was extracted from the capsules by dissolving in sodium dihydrogen phosphate buffer (10 mM, pH = 2.2) and ethanol, then made alkaline (pH = 9) and extracted using chloroform. The amount of codeine in AsthmaWan was found to be 61.8 μg/capsule (R.S.D. = 7.9%, $n = 9$). Excellent resolution was obtained despite the complexity of the product which claimed to contain at least nine herbal ingredients, none of which will give rise to codeine. As a further confirmation method, LC-MS-MS is accurate and specific. The LC method has been validated for linearity, limit of detection, limit of quantification, accuracy and specificity. Greater awareness of and control over undeclared drugs in complementary medicine are necessary to ensure patients' safety.

Keywords: Codeine; Chinese medicine; Reversed phase chromatography; HPLC; LC-MS-MS

1. Introduction

Chinese herbal medicine (CHM) and Chinese proprietary medicine (CPM) continue to be widely used by people throughout the world [1]. Assessment of the safety and efficacy of Chinese medicine is an important issue for the health profession. The adulteration of synthetic therapeutic substances of Traditional Chinese Medicine has been previously reported [2–7]. Conventional analytical methods include thin-layer chromatography (TLC) [4] and high performance liquid chromatography (HPLC) [5,6]. Being one of the most sensitive and specific methods for molecular

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analysis, mass spectrometry (MS) has an important place amongst the various spectrometric techniques [8]. Combining chromatography with mass spectrometry, such as liquid chromatography-mass spectrometry (LC-MS), offers the possibility of taking advantage of both chromatography as a separation method and mass spectrometry as an identification method.

The objective of this study was to develop accurate and sensitive methods to detect and quantify codeine present in a CPM for asthma.

2. Experimental

2.1. Chemicals

All chemicals used were analytical grade or better. Acetonitrile and methanol (HPLC grade) were purchased from Reagent Chemical Industry (Thailand). Water for HPLC was treated with a Milli-Q water purification system (Millipore, France). Codeine phosphate (BP) was used as a standard in HPLC determination. Codeine standard solution in methanol (1 mg/ml) obtained from Sigma (USA) was used as a standard in LC-MS-MS.

2.2. HPLC procedure

Hewlett Packard series 1050 quaternary gradient pump, photo-diode array detector, HP series 1100 autosampler and HP Lichrosorb reversed phase (RP) C18 200 mm × 4.6 mm, 10 μm column were used for gradient elution (acetonitrile/phosphate buffer). Solvent A = sodium dihydrogen phosphate buffer (25 mM, pH = 3.0), solvent B = acetonitrile; step gradient is from 5% of B to 30% of B over 45 min and then to 50% of B over 5 min and held for another 5 min. Total chromatography duration was 55 min. The equilibration time between two consecutive injections was set at 7 min (total cycle time 62 min). The flow-rate of mobile phase was 1 ml/min. Injection volume was 5 μl. The detection wavelength was set at 280 nm. The UV spectra from 200 to 400 nm were recorded online during the chromatographic run.

2.3. LC-MS-MS procedure

LC-MS-MS was performed on a Perkin Elmer PE-Sciex API 300 triple quadrupole ionspray mass spectrometer interfaced with a Shimadzu microbore high performance liquid chromatograph. Samples were separated on a Phenomenex Luna 5 μm RP-C18 (2) 50 mm × 1.0 mm column eluted at 50 μl/min using a binary pump system (Shimadzu LC-10 A). The mobile phase composition was (A) 1 mM ammonium acetate; (B) 95% of acetonitrile in water with 1 mM ammonium acetate. Sample injection volume was 5 μl. The initial mobile phase composition was 20% (B) for 3 min and a linear gradient to 100% (B) over 0.5 min, which was held for 3 min. The equilibration time between two injections was set at 5 min. The total chromatography duration was 11 min.

Mass spectra were acquired using the Perkin Elmer proprietary software LC2Tune 1.3 or Sample Control 1.3. Spectra were analyzed and integrated using BioMultiView 1.3. Positive precursor and product ions were acquired by infusion of standard codeine in 1.6 μg/ml methanol at 5 μl/min using a Harvard (Natick, MA) syringe infusion pump. Codeine was detected in positive mode using liquid chromatography-selected reaction monitoring mass spectrometry (LC-SRM MS). The mass spectrometer was used with ionspray and orifice voltages of 5000 and 36 V, respectively. The collision energy was 70 eV with nitrogen used as the collision, nebulizer and curtain gases. Codeine was detected by monitoring the transition of m/z 300.1 to 215.0. Blank injections were run between samples and standards at regular intervals to check the stability of the response.

2.4. Preparation of the sample

Blister packed green capsules of AsthmaWan (Yangcheng brand from China) in a white and green box were purchased from a Chinese medical hall. Contents of ten capsules were weighed and dissolved in 25 ml of sodium phosphate buffer 10 mM (pH = 2.2). After centrifugation, the supernatant was decanted, and 40 ml of ethanol was

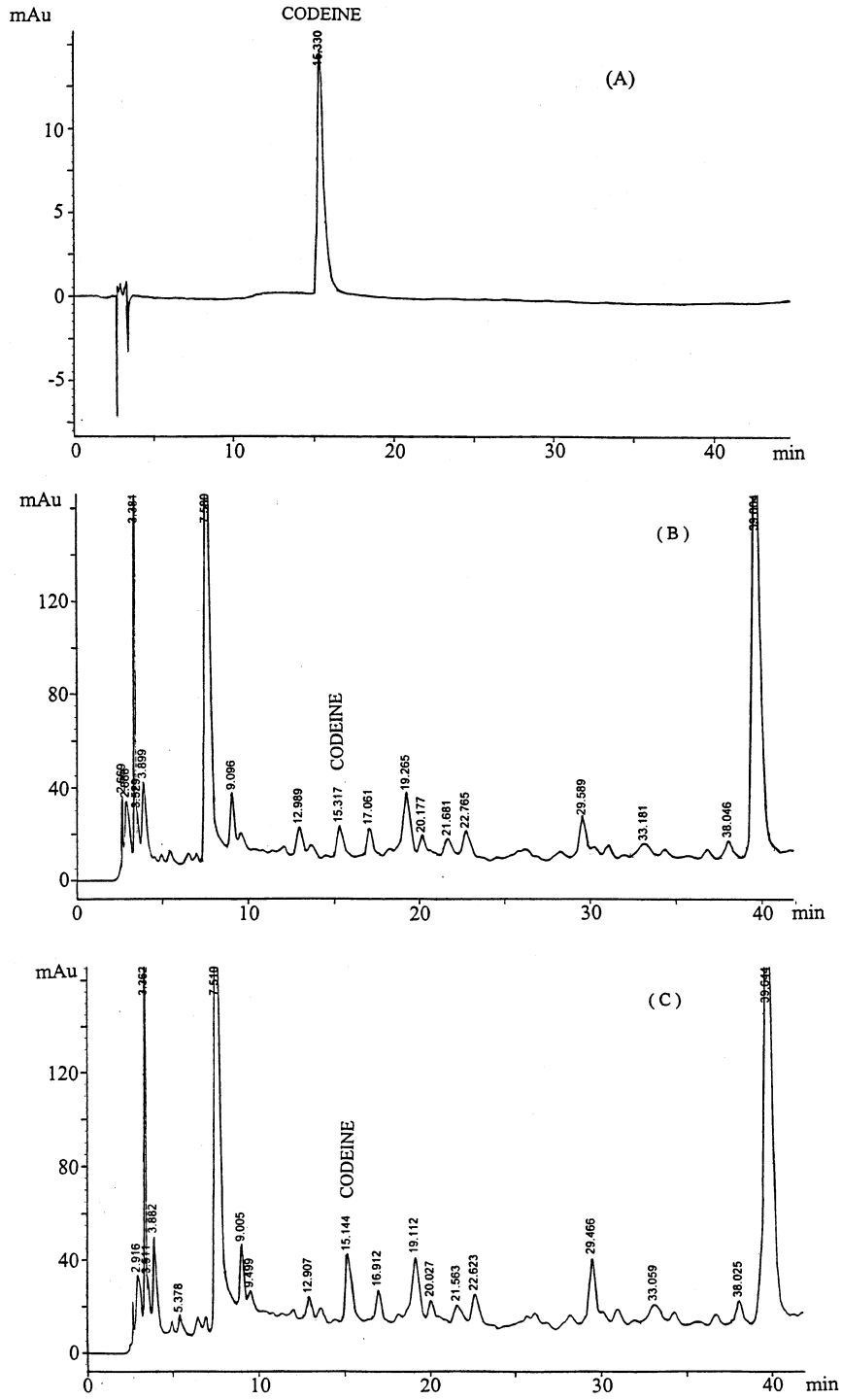

Fig. 1. RP-HPLC chromatograms from analysis of (A) standard codeine phosphate at 0.39 mg/ml, (B) extract of CPM AsthmaWan and (C) CPM extract with spiked codeine standard (0.78 mg standard codeine corresponding to 120% of the amount detected, was spiked in before extraction).

added to it. This mixture was then centrifuged and the supernatant was decanted and made alkaline using strong ammonium solution to pH = 9 and extracted using chloroform. The chloroform layer was evaporated to dryness by rotary evaporator and the residue was dissolved in 2 ml of methanol for analysis. The standard method [9] was followed in the recovery determination.

3. Results

3.1. Detection and quantification of codeine by HPLC

HPLC response was found to be linear over the concentration ranges examined for both samples and standards. The standard solution was prepared in methanol from 78 μg/ml to 1.0 mg/ml. The calibration curve obtained from six points was $y = 1164x - 25.29$, $r^2 = 0.999$. Four calibration curves were obtained on different days with R.S.D. = 3.93%. The amount of codeine in AsthmaWan was found to be 61.8 μg/capsule (R.S.D. = 7.9%, $n = 9$). Intra-day and inter-day repeatability of the retention time and peak area was excellent (R.S.D. < 2.6%, range 0.2–2.6%, $n = 6$). For the recovery study, 0.39, 0.78 and 1.17 mg of standard codeine, which corresponded to 60, 120 and 180% of the amount detected, were spiked in before extraction. The recovery of codeine, as determined by comparing the amount obtained and spiked, was 83.3% (range 79.1–88.5%, $n = 3$). The detection limit was found to be 44.0 μg/ml (signal-to-noise ratio was 3:1). The limit of quantification was found to be 60.0 μg/ml (signal-to-noise ratio was 10:1). The HPLC chromatograms of standard codeine, the CPM extract and the CPM extract spiked with codeine standard were shown in Fig. 1. The peak of interest was scanned from 200 to 400 nm and the UV profile was identical to that of the codeine standard.

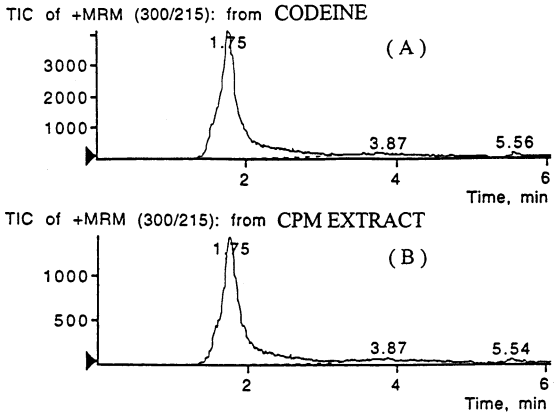

Fig. 2. LC-MS-MS chromatograms in the SRM mode of (A) codeine standard solution at 1.6 μg/ml, and (B) extract of CPM AsthmaWan.

3.2. Confirmation of codeine by LC-MS-MS

The codeine standard produced a positive ion of m/z 300.1 corresponding to the MH^+ precursor ion. Product ion scans of this precursor ion produced a fragmentation pattern dominated by an ion at m/z 215.0. The LC-SRM MS of codeine standard produced a peak at 1.8 min (Fig. 2). The presence of codeine from the extract of AsthmaWan was confirmed by detection of a similar peak at 1.8 min (Fig. 2).

4. Discussion

AsthmaWan is a capsule form of CPM, which contains about 0.25 g of dark brown powder of herbs in each capsule. It is claimed to indicate for all types of asthma. Information available on the product package varied from that on the product insert. Eleven herbal ingredients were stated on the product package while nine were stated on the product insert (Table 1), none of which will give rise to codeine. Despite the complicated herbal matrix, good resolution of the codeine peak was achieved using the HPLC method developed. Hence, no interference from other components was encountered in the chromatograms.

Codeine is one of the opioid alkaloids found in species of the Papaveraceae family [10]. It can be used as a cough suppressant (dosage 45–120 mg/day) and analgesic (dosage 120–240 mg/day) [11]. The manufacturer's recommended dose of AsthmaWan was two capsules, three times a day.

Table 1
The composition of AsthmaWan as stated on the product package and insert

No.	Ingredients of AsthmaWan	Percentage as stated on product insert (%)	Percentage as stated on package (%)	Presence on package or insert
1	Radix Codonopsis Pilosulae	10	10	Both
2	Folium Eriobotryae	15	10	Both
3	Rhizoma Et Radix Cynanchi Stauntoni	5	5	Both
4	Radix Glycyrrhizae	5	5	Both
5	Semen Armenicacae Amarae	10	10	Both
6	Cortex Mori Radicis	5	5	Both
7	Cudrania Cochinenisis	10	10	Both
8	Fructus Amomi	–	5	Package
9	Gecko	–	10	Package
10	Datura Metel L	–	5	Package
11	Ficus Simplicissima Lour	–	20	Package
12	Deng Lang Tsao	20	–	Insert
13	Mau Taur Tsao	20	–	Insert
	Total	100	95	

Taking the capsules according to the recommendation and according to the small amount of codeine found (61.8 μg/capsule) in this study, the dosage falls below the therapeutic daily dose of codeine for any therapeutic purposes. Thus, the reason for the presence of codeine in this product is not known. Lack of standardization, contamination, misidentification, incorrect labelling and adulteration are some of the common problems encountered with herbal medicine [12]. A report [3] in 1997 showed that 618 CPM samples (23.70%) among the 2609 samples tested were adulterated with unlabelled synthetic therapeutic substances.

The presence of an undeclared drug in the complex matrix of herbs offers a challenge in extraction, separation and detection. The described method for extraction and separation is based on the fact that alkaloids, such as codeine, can be extracted as a free base with organic solvents (e.g. chloroform) and as a protonated base with polar solvents (e.g. water) [10]. As the complex matrix is made up of at least nine different components (Table 1), it is not possible to prepare an identical matrix without the presence of codeine. All the samples obtained from various sources were found to contain codeine. Hence, no placebo was used in the analysis. The photo-diode array detector allows the UV spectrum of a peak to be acquired. By comparing the UV spectrum and the retention time of a peak with that of a standard drug, the identity of the drug can be determined. Thus, the method is specific.

Due to its high specificity and sensitivity, analytical methods based on combined liquid chromatography and mass spectrometry (e.g. LC-MS-MS) can be rapidly established to detect low levels of drugs in herbal medicine with complicated matrices. The transition from the potonated precursor ion to the product ion was used in the selected reaction monitoring (SRM). This method provides a further confirmation of the presence of codeine. To the authors' knowledge, this is the first report of an LC-SRM MS application in the analysis of codeine in CPM.

The CPM AsthmaWan in this study was readily available over-the-counter. The presence of undeclared codeine is potentially dangerous although the amount that has been found in this study is low. Regulation of herbal medicine, including control of proper labelling, monitoring of and fast screening for undeclared drugs, is necessary to ensure patients' safety.

5. Conclusions

A sensitive and accurate HPLC method for the detection and quantification of undeclared codeine in a CPM has been developed. The presence of codeine in a CPM is further confirmed using LC-MS-MS. The method has been validated for linearity, limit of detection, limit of quantification, accuracy and specificity. Greater awareness of and control over undeclared drugs in complementary medicine are necessary to ensure patients' safety.

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