Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 401–406
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography–mass spectrometry–mass spectrometry

Aik-Jiang Lau [a], Michael J. Holmes [b,c], Soo-On Woo [d], Hwee-Ling Koh [a,*]

[a] Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543
[b] Department of Biological Sciences, Faculty of Science, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260
[c] Tropical Marine Science Institute, National University of Singapore, 14 Kent Ridge Crescent, Singapore 119223
[d] Industrial Health Laboratory, Centre for Analytical Science, Health Sciences Authority, 11 Outram Road, Singapore 169078

Received 2 August 2002; received in revised form 11 November 2002; accepted 11 November 2002

Abstract

Adulterations with synthetic drugs are common problems with herbal medicine and this can potentially cause serious adverse effects. It is therefore important to determine the presence of synthetic drugs in herbal medicine to ensure patients' safety. The objective of this study was to develop sensitive and specific methods to analyse phenylbutazone, caffeine and oxyphenbutazone present in a traditional Indonesian herbal product. Liquid chromatography–mass spectrometry–mass spectrometry (LC–MS–MS) methods in the selected reaction-monitoring (SRM) mode were developed. It was found that the sample contained 0.53% w/w ($n = 3$, RSD = 7.56%) phenylbutazone and 0.04% w/w ($n = 3$, RSD = 8.39%) caffeine. This corresponded to 43.17 mg phenylbutazone and 3.23 mg caffeine in each sachet of powder. The methods were validated for linearity, precision, accuracy, LOD and LOQ. LOD and LOQ were found to be 3.69 and 12.29 ng/ml, respectively for phenylbutazone. For caffeine, the LOD and LOQ were 0.84 and 2.80 ng/ml, respectively. Oxyphenbutazone in the sample was found to be present at a level below the quantification level of 10.2 ng/ml. With better methods developed for analysis of adulterants in herbal medicine, the quality and safety of these medicines can be better controlled and regulated to ensure patients' safety.

Keywords: Liquid chromatography–mass spectrometry–mass spectrometry; Adulterants; Traditional herbal medicinal product; Quality control

1. Introduction

Herbal medicine, a form of complementary and alternative medicine (CAM), is becoming increasingly popular in both developing and developed countries [1]. With this increased usage, the safety,

* Corresponding author. Tel.: +65-6874-7962; fax: +65-6779-1554.
E-mail address: phakohhl@nus.edu.sg (H.-L. Koh).

0731-7085/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(02)00637-4

quality and efficacy of these medicines have been an important concern for health authorities and health professionals. Although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effects [2]. Adverse effects of herbal medicine may be due to factors such as adulteration, substitution, contamination, misidentification, lack of standardization, incorrect preparation and/or dosage, and inappropriate labelling and/or advertisement [2].

Adulterations with synthetic drugs and toxic heavy metals are major problems with herbal medicine [3–5]. Cases of clinical complications and adverse effects resulting from the synthetic drugs in herbal medicine have been reported [3,5,6]. The clinical consequences can be serious and sometimes life threatening. This is especially so when patients are on other similar medications, medications with potential interactions or when patients have other predisposing medical conditions. These incidences highlight the importance of detecting the presence of any synthetic drugs in herbal medicine to ensure their safety.

Analyses of adulterants using TLC [4,7,8], HPLC [8–11] and CE [12] have been reported. These methods generally cannot be used for high confidence identification. Tandem techniques such as GC–MS [8,9,13], LC–MS [8,10] and CE–MS [14,15] provide higher specificity and these have been used for confirmation purposes.

An Indonesian product of powdered herbs in a sachet, Serbuk Jarem (Encok), was initially screened for synthetic drugs using previously reported method [9]. It is claimed to treat conditions such as rheumatism, body ache and bone ache, muscle and joint pain, giddiness, toothache, backache, and chronic numbness. Three drugs, phenylbutazone, caffeine, and oxyphenbutazone, were detected. To date, quantification of synthetic drugs adulterated in herbal medicine using LC–MS–MS has not been reported. The objective of this study was to develop sensitive and specific liquid chromatography-selected reaction monitoring mass spectrometry (LC–SRM–MS) methods to analyse phenylbutazone, caffeine and oxyphenbutazone present in the complex matrix of a traditional herbal product.

2. Experimental

2.1. Materials

All reagents used were of analytical grade or better. Milli-Q (Millipore, France) water was used. Phenylbutazone, caffeine and oxyphenbutazone were ASEAN reference standards. The sample analysed was Serbuk Jarem (Encok) manufactured by P.J. Sari Alam, Cilacap, Indonesia.

2.2. Sample and standard preparation

Thirty milliliters of 96% ethanol was added to 2 g of the powdered sample. The suspension was heated to boiling and filtered. This extraction was repeated three times. The combined filtrate was evaporated to dryness. The residue was then dissolved in 2 ml of methanol and filtered. Three different extracts were separately prepared for quantification. Four concentrations of phenylbutazone (1–50 μg/ml) and caffeine (0.1–10 μg/ml) standard solutions were prepared. The sample and standard solutions were diluted using 80% v/v methanol and 20% mobile phase A (2 mM ammonium formate, 50 mM formic acid).

2.3. LC–SRM–MS method

A Perkin Elmer Sciex API 300 triple quadrupole turbo ionspray mass spectrometer (Ontario, Canada) interfaced with a Shimadzu LC-10AD binary pump (Japan) high performance liquid chromatograph was used. Samples were separated on a Phenomenex Luna C18(2) column (50×2 mm id, 5 μm) and eluted at a flow rate of 200 μl/min. The mobile phases were (A) water with 2 mM ammonium formate, 50 mM formic acid and (B) 95% v/v acetonitrile, 5% v/v water, with 2 mM ammonium formate, 50 mM formic acid. Sample injection volume was 5 μl. Different isocratic elutions were employed to separate the three drugs. Phenylbutazone was eluted isocratically with 40% A for 6 min while caffeine was eluted with 85% A for 5 min. Elution with 50% A for 5 min was used for the detection of oxyphenbutazone. Blank injections of 80% v/v methanol and 20% v/v mobile phase A were run between

samples. Triplicate injections of all the standards and samples were carried out. Standards were run intermittently to verify the instrument response.

Positive precursor and product ions were acquired by infusion of the standards in 80% v/v methanol and 20% v/v A at 10 μl/min using a Harvard (Natick, MA) syringe infusion pump. Phenylbutazone, caffeine and oxyphenbutazone in the samples were detected in the positive mode using LC–SRM–MS. For analysis of phenylbutazone and oxyphenbutazone, the mass spectrometer was used with turbo ionspray and orifice voltages of 4400 V and 31 V respectively. For analysis of caffeine, the turbo ionspray and orifice voltages were 4600 and 31 V, respectively. The collision energy for the analysis of phenylbutazone and caffeine was 25 eV with nitrogen used as the collision, nebulizer and curtain gas. Collision energy of 21 eV was used for the analysis of oxyphenbutazone. The turbo ionspray gas (thermally assisted evaporation) flowed at 7 l/min with the temperature set to 425 °C. Phenylbutazone was detected by monitoring the transition of m/z 309.3 to 120.0, and caffeine was detected by monitoring m/z 195.1 to 138.0. For oxyphenbutazone, the transition of m/z 325.1 to 204.0 was monitored.

3. Results

LC–SRM–MS analyses detected phenylbutazone, caffeine and oxyphenbutazone. Positive mode MS scans of phenylbutazone, caffeine and oxyphenbutazone produced $[M+H]^+$ product ions with m/z 309.3, 195.1 and 325.1 respectively. Product ion scans produced fragmentations dominated by ions at m/z 120.0, 138.0 and 204.0, respectively (Fig. 1) [14,16]. FIA of the three standards produced stable total ion currents for the SRM transition from the parent ions to the above fragment ions. The LC–SRM–MS total ion chromatograms of phenylbutazone, caffeine and the herbal sample are presented in Fig. 2.

Linear calibration curves of phenylbutazone and caffeine were obtained for the range 1–50 μg/ml ($r^2 > 0.9975$) and 0.1 μg/ml to 10 μg/ml ($r^2 > 0.9999$), respectively. Phenylbutazone (2.4 mg) and

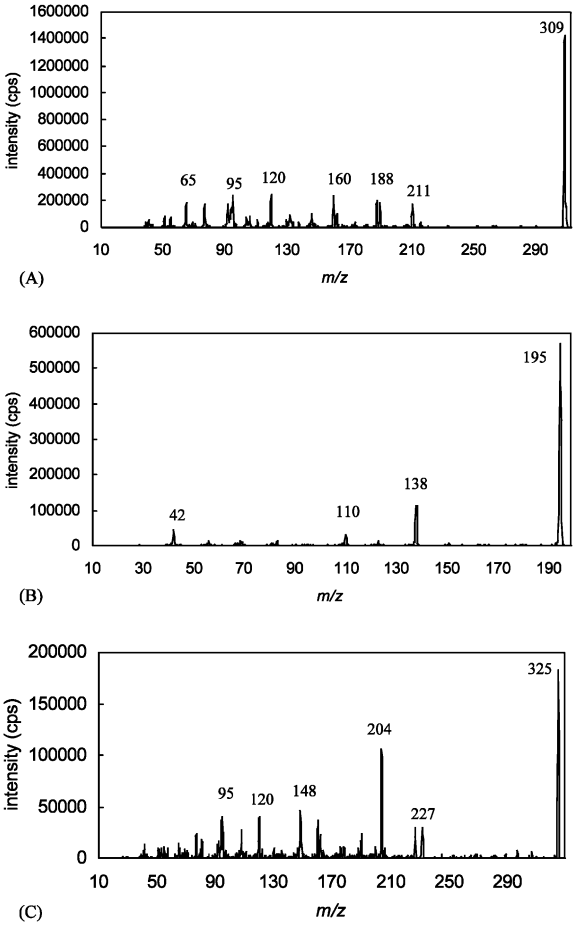

Fig. 1. MS–MS scans of (A) phenylbutazone, (B) caffeine and (C) oxyphenbutazone.

0.7 mg of caffeine were spiked into the sample before extraction. The recoveries were 108% ($n = 3$, RSD = 6.22%) and 106% ($n = 3$, RSD = 5.24%), respectively, validating the methods for these compounds. The concentration of phenylbutazone in the sample was found to be 0.53% w/w ($n = 3$, RSD = 7.56%) and the concentration of caffeine was 0.04% w/w ($n = 3$, RSD = 8.39%). Each sachet of the herbal product contained an average of 8.17 g ($n = 3$) of powder. Therefore, each sachet contained 43.17 mg phenylbutazone and 3.23 mg caffeine.

The instrument or injection precision was obtained by analysing the peak area variation of at least 10 injections of 4.750 μg/ml phenylbutazone

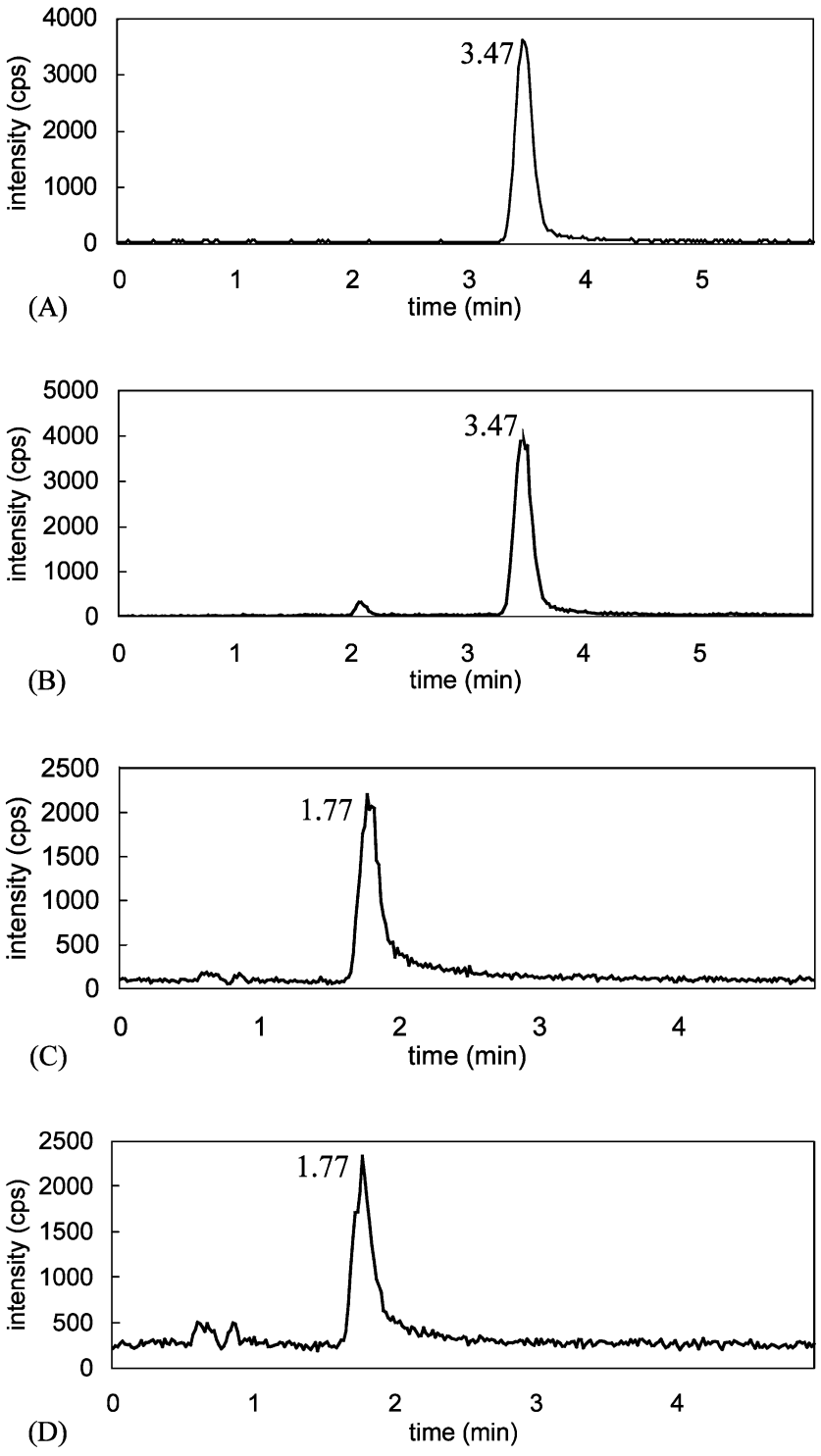
4000
3000
2000
1000
0
intensity (cps)
3.47
0
1
2
3
4
5
time (min)
(A)
5000
4000
3000
2000
1000
0
intensity (cps)
3.47
0
1
2
3
4
5
time (min)
(B)
2500
2000
1500
1000
500
0
intensity (cps)
1.77
0
1
2
3
4
time (min)
(C)
2500
2000
1500
1000
500
0
intensity (cps)
1.77
0
1
2
3
4
time (min)
(D)

Fig. 2

and 0.495 μg/ml of caffeine. The injection precision was found to be 4.2% ($n = 11$) for phenylbutazone and 10.3% ($n = 10$) for caffeine. The inter-day repeatablity (RSD) of phenylbutazone and caffeine were 4.4–8.9% ($n = 3$) and 0.7–17.0% ($n = 3$) respectively. The LOD and LOQ for phenylbutazone were 3.69 and 12.29 ng/ml, respectively. LOD and LOQ of caffeine were 0.84 and 2.80 ng/ml, respectively.

Spiking of a known concentration of oxyphenbutazone standard into the herbal extract gave a corresponding increase in the peak area of the oxyphenbutazone peak, confirming the presence of oxyphenbutazone. The detection (signal to noise ratio of 3:1) and quantification (signal to noise ratio of 10:1) level of oxyphenbutazone was 2.0 and 10.2 ng/ml, respectively. The concentration of oxyphenbutazone in the sample was found to be below the quantification level and near the detection level. Quantification of this drug was thus not attempted.

4. Discussion

The composition of Serbuk Jarem (Encok), as stated on the outer package, is *Myristicae semen* (10%), *Blumaeae folium* (30%), *Kaemferiae rhizoma* (20%), *Curcumae rhizoma* (30%) and *Retrofracti fructus* (10%). One of the herbal ingredients stated on the outer package was different from that stated on the sachet. *Zingiberis rhizoma* (30%) was stated on each sachet instead of *Blumaeae folium* (30%). This herbal product was recalled and banned in Singapore on 23 January 2002. Future batches, if found to be free from adulterants, may be allowed to be sold in Singapore. The manufacturer's recommended dose was one sachet twice daily (for treatment) or one sachet in 2–3 days (for 'well being'). According to the amount found in this study, these corresponded to a maximum daily dose of 86.34 mg phenylbutazone and 6.46 mg caffeine. The therapeutic dose of phenylbutazone for ankylosing spondylitis is initially 200 mg 2–3 times daily, and then the dose is reduced to a minimum, usually 100 mg two to three times daily [17]. For caffeine, the usual dose is 50–250 mg [18]. Therefore, the dosages of phenylbutazone and caffeine in the herbal product are below the therapeutic daily doses. However, if patients are concomitantly taking the same prescribed western drugs or other drugs of the same class, or have medical conditions that the drugs are contra-indicated, risks of adverse effects will increase.

The extraction method used in this study is simple, fast, efficient, and gives good recovery rates. Many herbal products contain multiple herbs and the analysis of drugs present in them is a challenging task. The use of a highly specific method such as SRM–MS is important to provide a confirmatory identification of the type of adulterants in the presence of potential interference by the complex matrix. Due to the numerous components present in herbal products, co-elution of the components may be a problem. HPLC methods are usually time-consuming in order to resolve the peaks sufficiently for quantification. The run times for these LC–SRM–MS methods are extremely short (5 to 6 min) compared to HPLC methods and they can be used for routine analysis. Besides, the MS detector is the most sensitive method of molecular analysis, with detection limits in the nanogram range achieved for these drugs. This is important especially for very potent drugs, as adulterants may be present in trace quantities. TLC may not be sensitive enough to detect adulterants. This work demonstrates the potentiality of LC–SRM–MS in the quality control of herbal medicine.

5. Conclusions

LC–SRM–MS methods have been developed for the analysis of phenylbutazone, caffeine and oxyphenbutazone from the complex matrix of a herbal product. These analytical methods are rapid, highly specific and sensitive. These have

Fig. 2. LC–SRM–MS total ion chromatograms of (A) 4.750 μg/ml phenylbutazone standard and (B) herbal sample analysed for phenylbutazone, (C) 0.495 μg/ml caffeine standard and (D) herbal sample analysed for caffeine.

been validated for linearity, precision, accuracy, LOD and LOQ. The presence of synthetic drug adulterants is potentially dangerous for patients. With better methods developed for analysis of adulterants in herbal medicine, their quality and safety can be better controlled and regulated to ensure patients' safety.

Acknowledgements

The authors gratefully acknowledge the financial support from the National University of Singapore Academic Research Fund and a research scholarship (A.-J. Lau). The technical assistance of Rasvinder Kaur d/o Nund Singh is appreciated.

References

[1] D.M. Eisenberg, R.B. Davis, S.L. Ettner, S. Appel, S. Wilkey, M.V. Rompay, R.C. Kessler, J. Am. Med. Assoc. 280 (1998) 1569–1575.
[2] G. Pinn, Aust. Fam. Phys. 30 (2001) 1070–1075.
[3] H.L. Koh, S.O. Woo, Drug Saf. 23 (2000) 351–362.
[4] W.F. Huang, K.C. Wen, M.L. Hsiao, J. Clin. Pharmacol. 37 (1997) 344–350.
[5] E. Ernst, Trends Pharmacol. Sci. 23 (2002) 136–139.
[6] E. Gertner, P.S. Marshall, D. Filandrinos, A.S. Potek, T.M. Smith, Arthritis Rheum. 38 (1995) 614–617.
[7] S. Yuen, C.A. Lau-Cam, J. Chromatogr. 329 (1985) 107–112.
[8] T. Cairns, E.G. Siegmund, B.R. Rader, Pharm. Res. 4 (1987) 126–129.
[9] S.Y. Liu, S.O. Woo, H.L. Koh, J. Pharm. Biomed. Anal. 24 (2001) 983–992.
[10] S.Y. Liu, S.O. Woo, M.J. Holmes, H.L. Koh, J. Pharm. Biomed. Anal. 22 (2000) 481–486.
[11] S.J. Lai, S.R. Binder, H. Essien, K.C. Wen, J. Liq. Chromatogr. 18 (1995) 2861–2875.
[12] Y.R. Ku, Y.S. Chang, K.C. Wen, L.K. Ho, J. Chromatogr. A 848 (1999) 537–543.
[13] A.M. Au, R. Ko, F.O. Boo, R. Hsu, G. Perez, Z. Yang, Bull. Environ. Contam. Toxicol. 65 (2000) 112–119.
[14] H.L. Cheng, M.C. Tseng, P.L. Tsai, G.R. Her, Rapid Commun. Mass Spectrom. 15 (2001) 1473–1480.
[15] Y.R. Chen, K.C. Wen, G.R. Her, J. Chromatogr. A 866 (2000) 273–280.
[16] R.A. Locock, R.E. Moskalyk, L.G. Chatten, L.M. Lundy, J. Pharm. Sci. 63 (1974) 1896–1901.
[17] British National Formulary, vol. 43, British Medical Association and the Royal Pharmaceutical Society of Great Britain, London, 2002.
[18] J.E.F. Reynolds, Martindale: the Extra Pharmacopoeia, 31st ed., Royal Pharmaceutical Society, London, 1996.