Mass spectral analysis of PC-SPES confirms the presence of diethylstilbestrol

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Introduction/objectives: *PC-SPES is an herbal mixture available over the counter for the treatment of prostate cancer. It was re-called in January 2002 due to alleged contamination with warfarin. Other laboratories, including our own, claim that the potent synthetic estrogen, diethylstilbestrol (DES) which has been used for many years to treat hormone dependent prostate cancer, could be detected in the herbal mixture. Recent clinical studies report objective responses in men with hormone dependent and naïve prostate cancer, and also describe isolated cases of estrogenic side effects. A lack of effective conventional treatments for advanced hormone refractory prostate cancer has led to a widespread use of PC-SPES by patients across the North America continent. The presence of DES in PC-SPES might explain both clinical response and observed side effects in men taking 6-9 capsules per day.*

Methods: *We tested five batches of commercially available PC-SPES using gas chromatography (GC) and high performance liquid chromatography (HPLC) upon methanolic extraction. Duplicate aliquots were tested for each batch and the results compared to standard curves generated using DES (99% purity).*

Results and conclusions: *We detected significant levels of DES in three out of five tested batches. The presence of a synthetic steroid in PC-SPES is not likely to have occurred as a result of its extraction from a herbal source. The implications of this finding highlight the necessity of regulated quality control and standardization of natural health products.*

Key Words: PC-SPES, alternative therapy, prostate cancer, diethylstilbestrol

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Introduction

PC-SPES (PC: prostate cancer - SPES: Latin for hope) is a herbal preparation recommended by naturopathic healers for the treatment of prostate cancer. It has been reported by the manufacture of the product to consist of the combined extracts of eight Chinese and American herbs: a) Isatis indigotica (da qing ye, Dyer's Woad), b) Glycyrrhiza glabra/glycyrrhiza uralensis (gan cao, Smooth/Common Licorice), c) Panax pseudo-Ginseng (san qi), d) Ganoderma lucidum (ling

zhi, Reishi), e) Scutellaria baicalensis (huang-qin, Baical Skullcap), f) Dendranthema (Chrysanthemum) morifolium Tzvel, g) Rabdosia rubesrens and h) Repenoa Serens (Saw Palmetto).

Many of these herbs in PC-SPES have been a part of Traditional Chinese Medicine (TCM) which has been mainstream therapy for hundreds of years in Chinese culture.[1] Studies of these herbs have revealed potent anti-cancer activity in test systems and suggest that their combined therapeutic effects are attributable to their individual mechanisms of action.[2] PC-SPES has been shown in pre-clinical studies to have potent activity in both *in vitro* and *in vivo* prostate cancer models.[2,3-8]

Several clinical studies have reported the use of PC-SPES to treat prostate cancer.[4,9-11] These studies differ with respect to the number of patients involved, stage of disease, the dosing regime applied for PC-SPES and the outcomes studied. These clinical reports all indicate a significant response among patients with either androgen dependent or androgen independent disease. The adverse reactions reported for PC-SPES (6 to 9 x 320 mg capsules daily) mirror those experienced by individuals taking the synthetic estrogen, diethylstilbestrol (DES) at a dose equivalent to approximately 1 mg per day. The estrogenic side-effects commonly include breast tenderness and/or enlargement and loss of libido and/or erectile dysfunction. Deep vein thrombosis, pulmonary embolus, myocardial infarction, and transient ischemic attack are reported in 15% of patients treated with low dose (1 mg per day) DES[12] and in 5% of patients using PC-SPES. DES was the first drug used for medical castration but fell out of favor due to its cardiotoxicity.[13] The clinical effects of PC-SPES and DES treatment are similar and include a reduction in serum PSA level >/ = 50% in most androgen dependent patients, a lowering of serum testosterone concentrations and a reduction in pain associated with metastasis. Androgen independent prostate cancer has also been shown to respond to treatment with synthetic estrogen.[14]

Up until recently, PC-SPES was available from BotanicLab Inc. (Brea. CA) in gelatin capsule form. According to the manufacturers assurance, the ethanolic extract of the capsular contents may be reproducibly characterized by high performance liquid chromatography (HPLC) due to the presence of six distinct peaks areas. The constituent(s) identities of these six peak areas have yet to be rigorously defined and their relative quantities may vary from batch to batch.[2]

In this research project we conducted both liquid chromatography (LC)- and gas chromatography (GC)-mass spectral (MS) analysis of methanolic extracts of PC-SPES. Five batches of PC-SPES were screened for DES, one of which previously had been reported by an FDA laboratory as testing negative. The analytical approach employed and test results from the mass spectral assays are described.

Experimental

Materials

Four batches of commercially available PC-SPES were purchased from a representative of Botaniclab Inc. in Vancouver, BC. One batch was donated by a patient attending Vancouver Mainland Prostate Cancer Support Group who was using the product. Table 1 describes their batch numbers and corresponding expiry dates. Four of these bottles were sealed upon arrival at the analytical laboratory and one bottle, which had been recovered from a prostate cancer patient, was unsealed. Diethylstilbesterol (99% purity) was purchased from Sigma Chemical Co. HPLC grade methanol (EM Science) was used for the extraction. HPLC grade Formic acid and Acetonitrile (EM Science) were used to prepare the mobile phase for LC-MS.

Standard and sample preparation

DES was dissolved in methanol for use as a chemical reference standard. The standard curve for the quantitation of DES in PC-SPES by LC with ultraviolet (UV) detection was prepared by serial dilution of the stock solution to the appropriate linear dynamic range. For each batch, the contents of 10 capsules were blended and homogenized. Duplicate aliquots of approximately 320 +/- 0.1 mg of PC-SPES material were weighed and extracted with 5.0 mL methanol using manual agitation and sonication. The tubes were centrifuged for 5 minutes at 2500 rpm and the contents cooled to ambient temperature. The methanolic extracts were then filtered through a 0.45 µm PTFE syringe filter prior to LC-MS and GC-MS analysis.

Gas chromatography

The HP Model 6890 Capillary Gas Chromatograph equipped with a Mass Selective Detector (GC/MS) and autosampler was used for qualitative analysis. Helium was the carrier gas used at a linear flow velocity of 37 cm/second. Separation was performed using an HP-5MS 5% Phenylmethyl Siloxane Capillary 30.0 m x 250 µm 0.25 µm nominal. The injection volume was 1.0 µL and samples were injected using the front inlet in splitless mode. The injector and detector temperatures were set at 250 and 280°C,

respectively. The oven temperature was held at 100°C for 3.00 minutes, raised to 310°C at 8°C /minute, and held for 5 minutes. The temperature was then rapidly elevated at 30°C /min to 320°C and this final temperature was held for 3 minutes.

High performance liquid chromatography

An Agilent 1100 series HPLC equipped with a Diode Array Detector (240 nm), Mass Selective Detector (Electrospray) and an autosampler was used for quantitative analysis. Mass Selective Detector settings were as follows: Mass range:100.00 to 500.00 m/z, Fragmentator: 50V, Gain: 1.0, Threshold: 10, Stepsize: 0.15. Spray Chamber conditions were as follows: Gas temperature: 350°C, Vaporizer: 425°C, Drying gas flow: 13.0 L/min, Nebulizer pressure: 60 psig, Quad temperature: 0°C, Vcap: 5000 V. Separation was performed using an Hewlett Packard Zorbax C18, 4.6 x 150 mm, 5 µm column at 35°C. The sample injection volume was 10 ul with a flow rate of 1.0 mL/minute. The mobile phase consisted of 0.1% formic acid in HPLC grade water (A) and acetonitrile (B). The mobile phase was isocratic using 45% B for 12 minutes at which point 100% B was introduced over 0.1 minute. The column was then washed using 100% B for 4.9 minutes and re-equilibrated to isocratic conditions for subsequent analyses. The total run time was 17 minutes with a re-equilibration time of 4 minutes.

Results

Five separate batches of PC-SPES were screened for the presence of DES using GC mass spectral analysis. Their allocated batch numbers and expiry dates are summarized in Table 1. The retention times for the two isomers of DES were matched to peaks in the total ion chromatograms (TIC) of ethanol extracts of PC-SPES. Figure 1 illustrates a TIC of DES in methanol compared to the TIC of the ethanol extract of PC-SPES lot #5430171.

Mass spectra were generated for peaks using TIC elution at retention times corresponding to the DES reference solution. The mass spectra obtained for the PC-SPES samples were directly compared with the mass spectrum for DES obtained from our reference solution as well as a reference library spectrum obtained from the NIST 98 mass spectral database. DES was detected and positively identified in three

TABLE 1. **Expiry dates and batches tested for DES contamination**

Batch number	Expiry date	DES detected (yes/no)
#5430171	07/2002	yes
#5439174	08/2002	yes
#5430193	09/2002	yes
#5431249	09/2003	no
#5431219	08/2003	no

The date of expiration is 2 years following manufacture

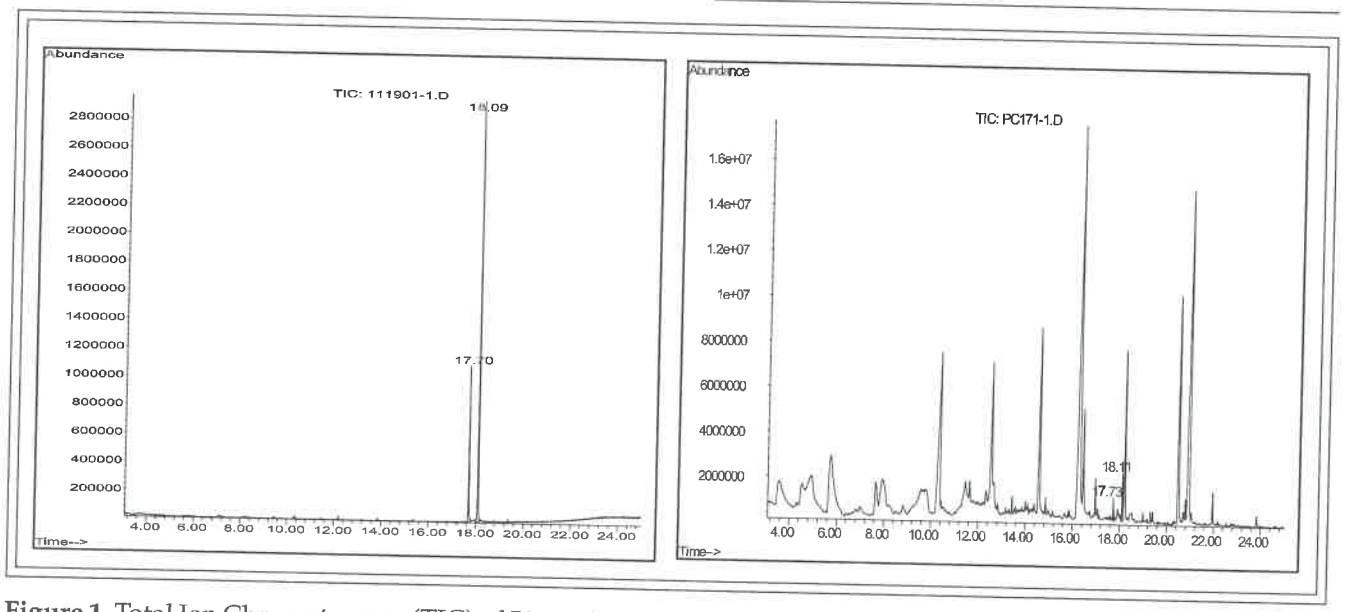

Figure 1. Total Ion Chromatogram (TIC) of 50 mg/ml diethylstilbesterol (DES) in methanol (left) compared to the TIC for 325 mg PC-SPES lot #5430171 extracted in 5ml MeOH (right).

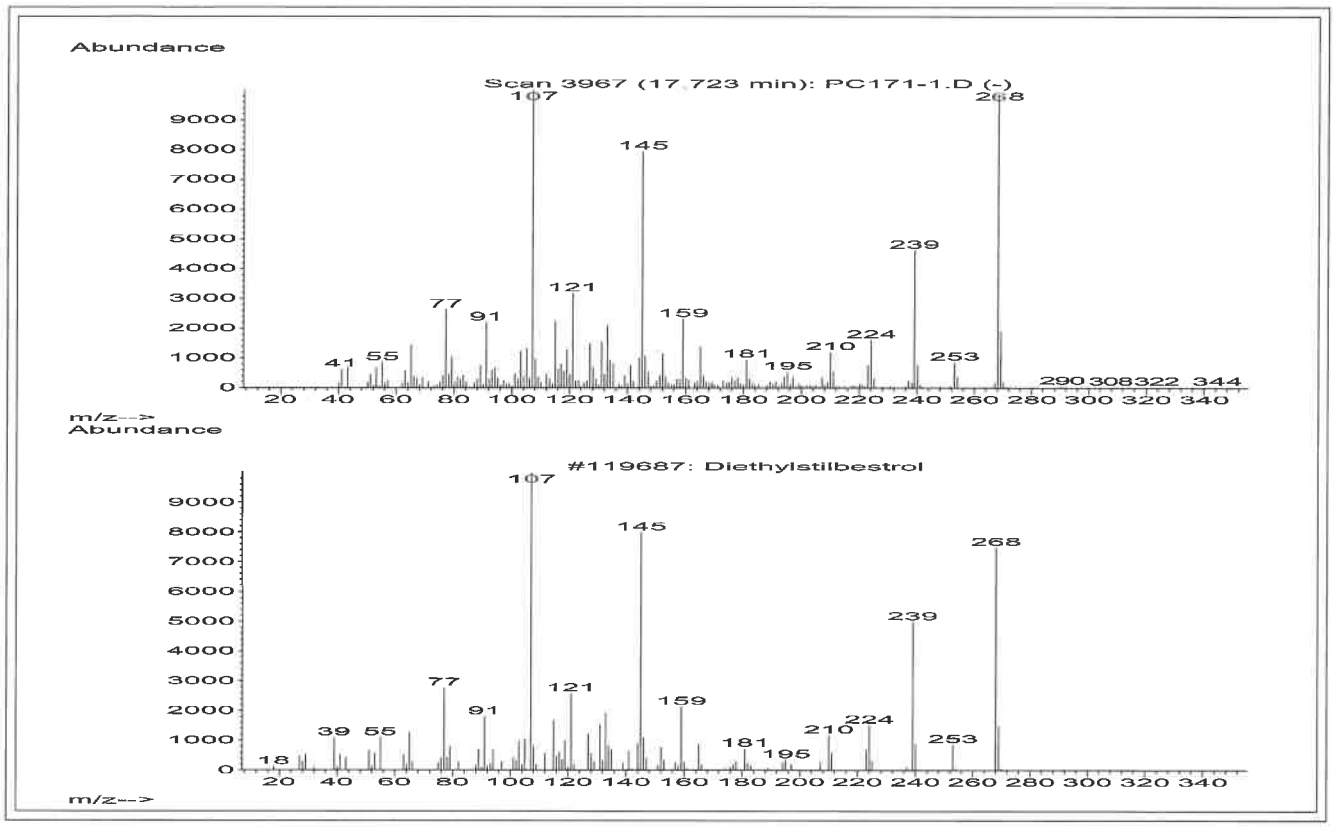

Figure 2. Comparison between the mass spectrum determined for peak eluting with retention time 17.72 minutes in the Total Ion Chromatogram (TIC) for PC-SPES lot #5430171 (325mg/5ml methanol) and the NIST 98 library catalogued mass spectrum for DES.

out of the five batches tested. Figure 2 illustrates a comparison between the mass spectrum of the peak eluting at 17.72 minutes in the TIC of PC-SPES lot #5430171 as directly compared with the NIST standard spectrum for DES. A search using the NIST mass spectral database generated a quality match of 99% for these spectra indicting that DES had been positively identified.

Using LC-MS we quantitatively analyzed lots of PC-SPES which had tested positive using GC-MS. The limit of detection (LOD) for DES detection using this method was 0.10 µg/mL and the determined limit of quantitation (LOQ) was 0.34 µg/mL (99% confidence). PC-SPES lots #5430171 and #5430193 were found to contain 20.79 and 3.51 µg DES/mean capsule. DES in lot #5439174 could not be quantitated. Lots #5431249 and #5431219 of the herbal did not contain DES at a detectable level using the LC-MS method described.

Conclusions

In September 2001 The Rocky Mountain Laboratory, an independent testing facility in the United States, reported a finding that DES was present in two batches of PC-SPES.[15] Botaniclab Inc. promptly recalled the two batches tested and launched an investigation into the finding. These two batches (lot #5430125 and lot #5436285) were different from the batches which were tested in our laboratory and we also discovered DES content in three out of five other batches. One of the batches which we tested positive for DES was lot #5430171. This same product was previously tested by an FDA approved laboratory in 2001 but no DES had been detected. This discrepancy between our findings and the FDA report appear to be due to the differences in analytical technique employed by the two laboratories. The FDA laboratory conducted LC-MS on a mixture containing 10 PC-SPES capsules (320 mg per capsule). From this mixture 0.500 g was extracted in 50 mL of ethanol:water (1:1) which would prepare an extract representing 32 mL of solvent per capsule. The estimated limit of detection of the testing facility reported was 0.0563 mg/mL which, when linearly translated, limits detection only to that greater than 0.2815 mg (281.5 µg) per capsule. This limitation indicates that a PC-SPES dose, taken by a self

administering patient, of nine capsules per day would have to total 2.5335 mg DES content before it would be detected using this technique.

The analytical technique employed by our laboratory detected 20.79 and 3.51 µg PC-SPES per capsule in two batches and detected trace quantities of DES in a third. If the tested batch of PC-SPES capsules are ingested at quantities recommended by Botaniclab Inc. for prostate cancer treatment (6-9 capsules) these amounts equate to daily doses of DES ranging from 31.59 –187.11 µg/day. The implications of this finding suggest that, although the origin of the DES remains unknown, many users of this herbal product have been unknowingly self-administering a potent synthetic estrogen alongside the battery of herbal constituents reported to be present in PC-SPES (including potent phytoestrogen). If PC-SPES contaminated with DES was used in the published clinical studies, it might be rational to suggest that the clinical efficacy observed in patients using this herbal product was at least in part due to DES.

In conclusion our findings indicate that patients who self administer PC-SPES have been inadvertently taking a standard form of medical castration therapy. These findings cast a shadow of doubt on pre-clinical results obtained thus far which describe the therapeutic activity of PC-SPES. PC-SPES is not currently available from the manufacturer as an alternative therapy for prostate cancer and the authors would like to emphasize that in our current unregulated natural products climate, quality control issues are a constant threat in the use of herbal treatments. □

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