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Short Communication

Simultaneous, stability-indicating capillary gas chromatographic assay for benzocaine and the two principal benzyl esters of Balsam Peru formulated in a topical ointment

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ABSTRACT

A capillary gas chromatographic assay is presented for the determination of benzocaine and benzyl esters of Balsam Peru in a pharmaceutical preparation. The method is stability-indicating and allows for simultaneous assay of benzocaine in the presence of the non-polar Balsam esters.

INTRODUCTION

Balsam Peru, a viscous brown exude collected from trunks of the tree *Myroxylon pereirae*, has been variously used in the treatment of skin wounds and other dermatological ailments, and in the perfume and confectionery industries [1,2]. It is a complex mixture of aromatic esters and alcohols including benzyl and methyl benzoate and cinnamate, styracin, nerolidal, vanillin, and benzoic and cinnamic acids [3]. Balsam Peru levels in a sample can be conveniently monitored by quantitation of benzyl benzoate and benzyl cinnamate esters. These esters comprise the cinnamein content and include benzyl benzoate, benzyl cinnamate, cinnamyl cinnamate and peruviol, and are generally around 50% (w/w) of the balsam [1].

Literature reports have centered on packed-column gas chromatographic (GC) analysis of Balsam Peru [4,5]. There have been no reports published using wall-coated capillary GC columns. More recently, a paper described high-performance liquid chromatography (HPLC) of benzocaine and benzyl benzoate in a topical preparation [6]. Our efforts utilizing liquid chromatography to assay benzocaine in combination with Balsam Peru were unsuccessful due to the plethora of small peaks arising from the balsam that coelute with benzocaine. Resolution of benzocaine from these interfering peaks would have resulted in a lengthy analysis time for the non-polar benzyl esters and a corresponding loss of efficiency. GC has the advantage of separation by boiling points, and GC capillary analysis offers higher theoretical plate counts and efficien-

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cies. The high resolving power obtained with capillary columns is a distinct and powerful advantage over packed-column GC and HPLC in resolving components of complex mixtures.

This paper describes a capillary GC analysis of the benzyl esters of Balsam Peru and benzocaine (*p*-aminoethyl benzoate) in a topical ointment. The high resolving power of capillary GC offers wide application of the method to a variety of different sample matrices containing these compounds.

EXPERIMENTAL

Apparatus

The gas chromatograph was a Hewlett-Packard 5890 with a flame ionization detector and was equipped with a 7673A autosampler (Hewlett-Packard, Paramus, NJ, USA). The column was a Hewlett-Packard HP-1, 50 m × 0.2 mm I.D., with 0.5 μm film thickness of cross-linked methyl silicone. Peaks areas were collected and measured using Beckman CALS PeakPro software (Beckman Instruments, Allandale, NJ, USA).

Reagents

All the reagents used were ACS reagent grade. Benzocaine and benzyl benzoate were USP reference standards (United States Pharmacopeia, Rockville, MD, USA). Benzyl cinnamate and benzophenone were purchased from Aldrich (Milwaukee, WI, USA). The purity of benzyl cinnamate was established at 99.4% using three independent techniques as follows: reversed-phase HPLC with area normalization assay, differential scanning calorimetry and reversed-phase thin-layer chromatography. The USP materials were used at 100% purity.

Chromatographic conditions

The injector temperature was 280°C, and the detector was set at 250°C. The column temperature was programmed at 140°C for 10 min then ramped at 2°C/min to a final temperature of 240°C and held for 15 min. The injection volume was 2 μl. The injection port was equipped with a split–splitless glass sleeve obtained from Hewlett-Packard containing silanized glass wool. A split ratio of 20:1 was established at 0.03 min following injection. Flow through the capillary column was measured using an electronic flow meter and found to be 0.5 ml/min. The column head pressure was 28 p.s.i. The septum purge flow-rate was set to approximately 2 ml/min and the split vent flow-rate was determined to be *ca.* 10 ml/min. Helium, hydrogen and air used for the carrier and flame were all zero grade to ensure absence of spurious peaks and baseline noise.

Standard preparation

The benzophenone internal standard solution was prepared by dissolving 2.0 g in 200 ml of chloroform. A working standard was made by accurately weighing approximately 15 mg of benzyl cinnamate, 30 mg of benzyl benzoate and 100 mg of benzocaine into a 100-ml volumetric flask. Exactly 5 ml of internal standard were pipetted into the flask, then the standard was brought to volume using chloroform.

Sample preparation

Samples of ointment equivalent to approximately 15 mg of benzyl cinnamate, 30 mg of benzyl benzoate and 100 mg of benzocaine were accurately weighed into 250-ml Erlenmeyer flasks. About 95 ml of chloroform were added, then exactly 5 ml of internal standard solution were added by transfer pipet. The samples were shaken on a wrist shaker until thoroughly dispersed. Portions of the samples were filtered through 0.45-μm PTFE filters prior to injection into the gas chromatograph.

RESULTS AND DISCUSSION

Detector response linearity was ascertained by making duplicate injections of six different standard solutions prepared in the following concentration ranges: benzyl benzoate, 0.1840–0.4906 mg/ml; benzyl cinnamate, 0.0918–0.2449 mg/ml; and benzocaine, 0.5996–1.5990 mg/ml. The correlation coefficients for the three compounds were 0.9999, 0.9994 and 0.9997, respectively.

Reproducibility of the chromatographic system was assessed by injecting a standard preparation six times and measuring the peak area ratios. The mean peak-area ratios were 0.734, 0.240 and 1.1198 for benzyl benzoate, benzyl cinnamate, and benzocaine, with relative standard deviations of 0.1, 0.9 and 0.3%, respectively. Using the conditions described, the column developed approximately 18 500 theoretical plates/m. The capacity factors

(k') were 8.4, 9.5, 11.4 and 16.0 for benzocaine, benzophenone, benzyl benzoate, and benzyl cinnamate, respectively.

Recovery studies were conducted by spiking placebo ointment with benzyl benzoate, benzyl cinnamate and benzocaine at 80, 100 and 120% of the label claim for each analyte. The amounts of analyte added for each spiking level in the order benzyl benzoate, benzyl cinnamate and benzocaine, were as follows: 80% label claim, 4.8, 2.4 and 16 mg/g of placebo ointment; 100% label claim, 6, 3 and 20 mg/g; 120% label claim, 7.2, 3.6 and 24 mg/g. The average recovery for benzyl benzoate, benzyl cinnamate and benzocaine for the three spiking levels was 99.8, 100.1 and 99.7% with relative standard deviations of 0.4, 0.8 and 0.8%, respectively.

A typical sample chromatogram, depicted in Fig. 1, clearly shows the separation of the sample components from small excipient peaks. This is a stability-indicating method for the analytes and was demonstrated in the following manner: Aliquots of the analytes dissolved in methanol were treated with either 10 mmol of sodium hydroxide or 10 ml of 30% hydrogen peroxide. The solutions were then heated at 50°C for 1 h. Chromatograms of the stressed samples were compared to sample and standard chromatograms. None of the degradation products generated under these conditions interfered with the analysis of the analytes. Quantitation of the resulting chromatograms confirmed the loss of the peaks of interest.

The hydrolysis product of benzocaine is *p*-aminobenzoate. The benzyl esters undoubtedly cleave at the ester linkage. These resulting products would have lower boiling points and would be expected to elute much faster from this column. A typical alka-

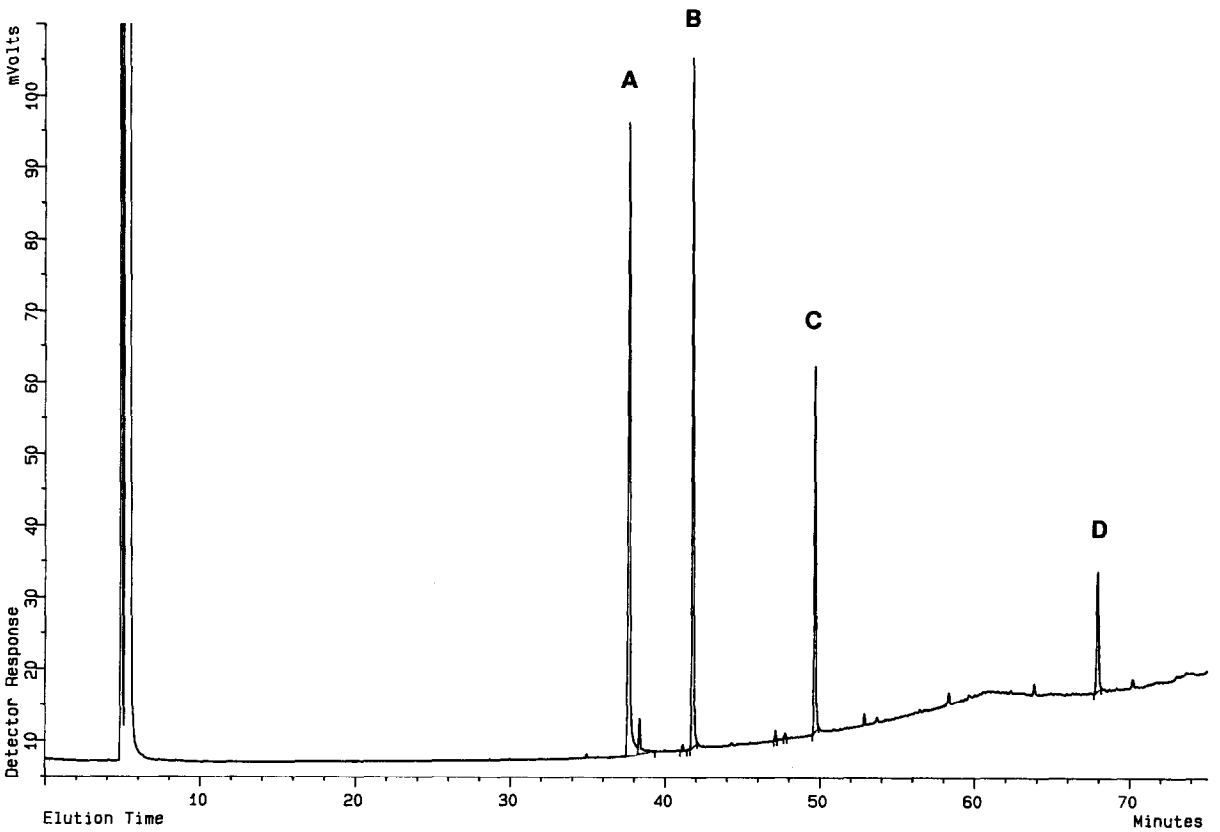

Fig. 1. Capillary gas chromatogram of an ointment sample preparation containing benzocaine and Balsam Peru. Peaks: A = benzocaine; B = benzophenone; C = benzyl benzoate; D = benzyl cinnamate.

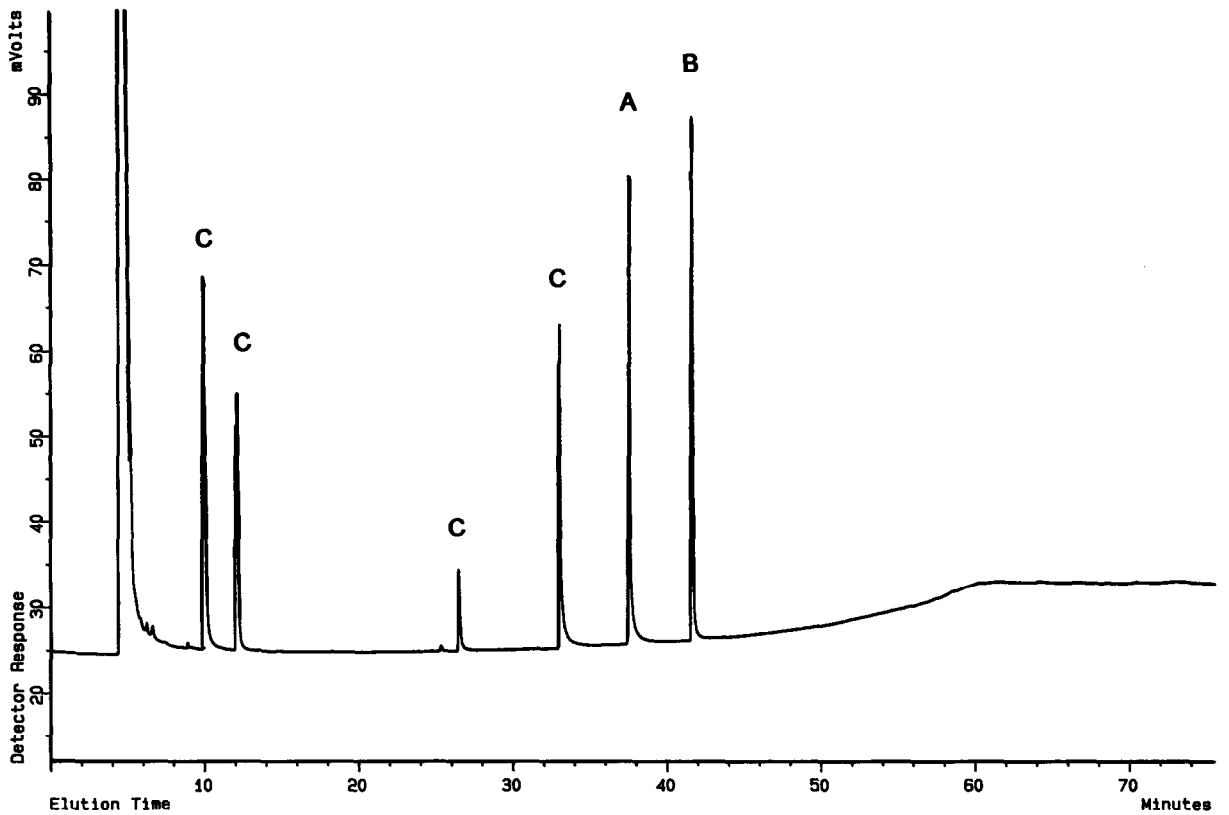

Fig. 2. Capillary gas chromatogram of an alkali-stressed standard solution showing loss of benzocaine, benzyl benzoate and benzyl cinnamate. Peaks: A = benzocaine; B = benzophenone; C = degradation product.

li-stressed standard solution is depicted in Fig. 2 which shows loss of the benzyl esters and the elution of degradation products early in the chromatogram. Quantitation of the remaining benzocaine peak confirmed the loss of this analyte.

While the lengthy run time of the analysis may appear unattractive for repetitive, routine sample quantitation, the complex nature of the Balsam Peru dictated the conditions to achieve separation of the analytes. Computer enlargement of the baseline showed numerous small peaks from an injected sample of Balsam Peru. The desired separation of the peaks of interest could be obtained only by choosing a system having high theoretical plate counts and efficiency. The column temperature was ramped slowly to achieve the desired resolution of the peaks of interest.

REFERENCES

1 S. Harvey, in A. Gennaro (Editor), *Remingtons Pharmaceutical Science*, Mack Publishing Co., Easton, PA, 18th ed., 1990, p. 765.
2 G. T. Walker, *Perfume Essent. Oil Rec.*, 59 (1968) 705–707.
3 H. Glash and H. Wagner, *Deutsch. Apoth. Ztg.*, 114 (1974) 45–49.
4 B. Mandrou, H. Mattras and S. Brun, *Trav. Soc. Pharm. Montpellier*, 38 (1978) 257–271.
5 A. Monard and A. Grenier, *5th Int. Symp. Chromatogr. Electrophoresis, Ann Arbor, 1968*, Humphrey Scientific Publishers, Ann Arbor, MI, 1969, pp. 529–536.
6 B. Gigante, A. Barros, A. Teixeira and M. J. Marcelo-Curto, *J. Chromatogr.*, 549 (1991) 217–220.