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HIGH-TEMPERATURE QUANTITATIVE GLASS CAPILLARY GAS CHROMATOGRAPHY

ANALYSIS OF PIPERINE AND OF QUININE–QUINIDINE

M. VERZELE, G. REDANT, S. QURESHI and P. SANDRA

Laboratory of Organic Chemistry, State University of Ghent, Krijgslaan 271 (S.4), B-9000 Ghent (Belgium)

SUMMARY

Different sampling methods for capillary gas chromatography have been evaluated for high-temperature work. On-column injection at low temperatures and moving-needle injection proved to be the best methods for obtaining reproducible and accurate results. The potentials of these two techniques are illustrated with examples of important analyses, *viz.*, piperine in pepper and quinine–quinidine in pharmaceutical preparations.

INTRODUCTION

Over the years it has become clear that improvements in quantitative capillary gas chromatography (CGC) are dependent on improvements in injection techniques[1]. The discrimination of splitting devices, for example, is well established. Flash evaporation of the sample solvent in vaporization chambers with split–splitless systems, direct injection, moving-needle injection, pre-column injection, etc., lead to systematic errors. Fractionation of the sample in the syringe needle by flash evaporation has recently been discussed[2,3].

At present, the introduction of a liquid plug into the column via a "cold on-column" injection technique seems to be the best method for achieving improved quantitative capillary GC. Two such techniques have been described in the literature, the direct sampling technique according to Schomburg *et al.*[4] and the on-column technique according to Grob and Grob[5]. On-column injection was devised in 1963, when Zlatkis and Walker[6] described direct sample introduction on to wide-bore steel capillary columns. In 1972, we showed that on-column injection does improve quantitative results[7]. This technique was also applied succesfully by Badings *et al.*[8] and in 1975 we described simple all-glass devices for on-column injection in everyday capillary GC[9]. Our observations appear to have passed unnoticed, and in recent contributions the on-column injection technique was "rediscovered"[10,11].

Although "on-column" injection has been commercially available since 1974[12], it turned out that even with this technology, quantitation was often erratic and more difficult than was generally recognized. The reasons why we were successful with

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on-column injection lie in the fact that only small amounts (0.05–0.5 μl) of highly concentrated solutions were rapidly (1 sec) injected on to wide-bore columns. The high carrier gas flow-rate, low inlet pressure, high permeability and low resistance of wide-bore columns resulted in rapid transfer into the capillary column with minimal losses due to back-flushing towards the septum. At that time, however, we were unaware of the possibilities of our system to give even better results. The necessary procedure, as has now been pointed out by Grob and Grob[5], was to inject at low temperatures. The same technique can be used to perform on-column injection at low temperatures if the injector body is not heated and the column is kept at a temperature below the boiling point of the solvent. The idea of on-column injection at low temperatures was advanced by Desty in 1965[13]. He noted that the ideal means of introducing high-boiling and thermolabile compounds is to inject very small amounts directly on to the column at the lowest possible temperatures, *e.g.*, that of the column itself.

The main feature of this technique is that the sample is present in the column before evaporation starts. The solvent, which is the major source of difficulties in evaporation injectors, is flushed into the column before evaporation of the compounds takes place. On-column injection at low temperatures means that the sample is injected into the column as a liquid. Any evaporation of the sample out of the syringe needle will cause discrimination and therefore the injector and GC oven temperature must be lower than the boiling point of the solvent. For many applications this is not practical. This problem has been studied by Galli *et al.*[3], and the installation of a secondary cooling system at the point where injection takes place ensures the introduction of a liquid plug even at GC oven temperatures higher than the boiling point of the solvent.

On-column injection at low temperatures is not always applicable, however, as at low temperatures some high-temperature stationary phases (*e.g.*, RSL-110, -903 and -702) are still solid and therefore cannot be used. In this work this was the case for RSL-903, which is a highly polar polyaromatic sulphone with a useful temperature range of 220–500°C, which was the only stationary phase that would separate quinine and quinidine conveniently. In such instances the falling-needle introduced by Van den Berg and Cox[14] appears to be applicable as the solvent is evaporated before the needle injects the sample.

The moving-needle injector is considered as a vaporizing injector. When the construction is such, however, that the inner diameter of the injector outlet is the same as that of the capillary column (Fig. 1a), the injector outlet is part of the column and in fact evaporation takes place in the column (on-column injection). With wide-bore columns even the construction shown in Fig. 1b is applicable. By introducing the needle at a high oven temperature, flash evaporation occurs and the needle can be retracted after a few seconds. Alternatively, the needle can be introduced at low column temperatures and evaporation occurs depending on the column temperature (the needle remains in the column during the analysis). This is then a cold on-column injection technique. The moving-needle injector is, of course, useful only for high-boiling compounds and is therefore a preferred injection technique for high-temperature capillary GC. With the construction shown in Fig. 1 the relative standard deviation for quantitative measurements is less than 1%. It should be noted, however, that for several applications, *e.g.*, pesticide analysis and steroid profiling,

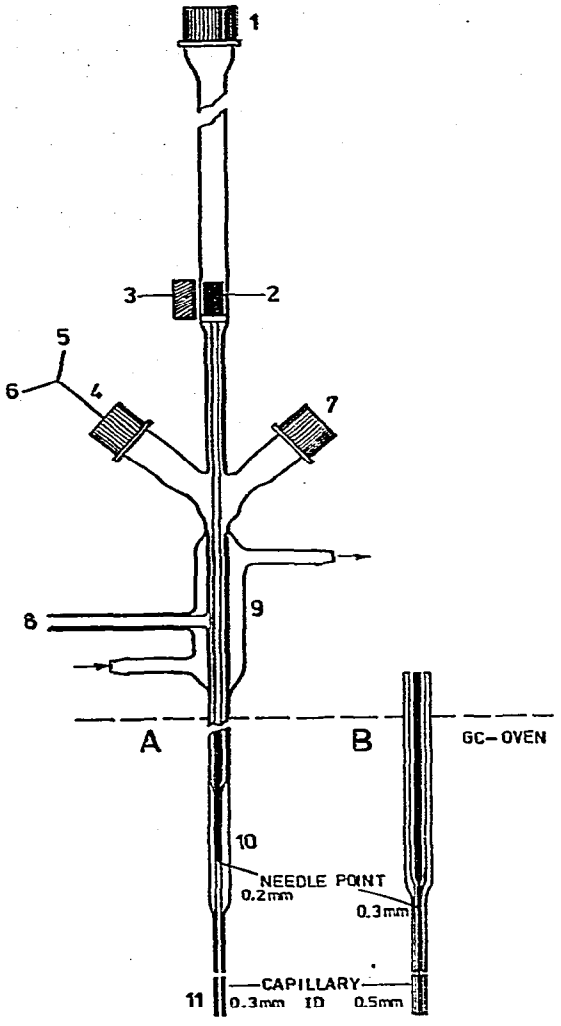


Fig. 1. Falling-needle injector adapted for high-temperature quantitative CGC. 1 = Screw-cap to withdraw needle for cleaning; 2 = iron plunger; 3 = magnet; 4 = solvent purge; 5 = capillary restriction or 6 = needle valve; 7 = injection port; 8 = carrier gas inlet; 9 = cooling jacket; 10 = capillary tubing, 6 mm O.D., 0.3 mm I.D., drawn to a point without restriction in I.D.; 11 = capillary column. For details of A and B for achieving on-column (hot or cold) injection, see text.

cooling of the gas stream is necessary in order to obtain accurate and reproducible results.

APPLICATIONS

Cold on-column injection applied to the determination of piperine in pepper and pepper extracts

Piperine is the single pungent principle of pepper and pepper extracts and its chromatographic analysis should replace total photometric concentration or total

nitrogen determinations. We have described several high-performance liquid chromatographic (HPLC) methods for piperine[15–17] but glass capillary separation has a higher efficiency and achieves a better separation. The procedure is as follows.

Reagents. Dichloromethane and methanol were dried and distilled. Pure piperine (m.p. 127°C) was obtained by extracting ground pepper with dichloromethane and recrystallization from methanol–water (1:1). The internal standard (tetrahydropiperine) was synthesized by catalytic hydrogenation. To 1 g of piperine in 50 ml of methanol were added 25 mg of platinum oxide and hydrogen at atmospheric pressure was passed above the stirred solution. The product (m.p. 40°C) was recrystallized from methanol–water (1:1).

Extraction of piperine from pepper and pepper extract. To about 70 mg of ground pepper or 10 mg of pepper extract were added 25 ml of dichloromethane and 25 ml of internal standard solution (0.104 mg/ml). After shaking, the samples were allowed to settle (in the dark to avoid photoisomerization of piperine), and 0.5 μl was injected.

Capillary gas chromatography. A Carlo Erba Fractovap 4160 gas chromatograph equipped with a Varian CDS-111 integrator was used. The CGC analyses were performed on a 25 m × 0.5 mm I.D. capillary column deactivated by high-temperature silylation and coated with OV-1. High-temperature silylation was carried out by a modified version[18] of Grob *et al.*'s technique[19]. The samples were injected by the cold on-column injection technique[3,5]; 0.5 μl was injected quickly at an oven temperature of 100°C with the secondary cooling on. After introduction of the sample the column temperature was immediately increased to 250°C.

Results and discussion. Injection has to be carried out at reduced temperature (100°C). Secondary cooling with air is not powerful enough to allow injection at the analysis temperature (250°C). That injection can be effected at 100°C is remarkable as the solvent dichloromethane is very volatile. With less volatile solvents (boiling point greater than 100°C) secondary cooling is unnecessary. Secondary cooling is also not required if the oven temperature is decreased to 35°C. From 250°C this takes a long time and is therefore unsuitable.

Chromatography of pure piperine (0.435–0.027 mg) against a constant amount of tetrahydropiperine gives a straight-line calibration graph passing through the origin. The response factors for piperine and tetrahydropiperine are virtually identical. A chromatographic trace of pure piperine with added internal standard is shown in Fig. 2A. By replicate analyses (n=6) of this sample the relative standard deviation of the on-column injection technique was found to be 0.93%. The relative standard deviation for a pepper sample (n=5) was 1.03%. Some results of determinations of the piperine content in pepper and pepper extracts are given in Table I. For comparison, data obtained with HPLC on nitrated–sulphonated phenylsilica are included[17].

The results of the CGC analysis are slightly higher than those obtained by HPLC. This can be attributed to the ideal peak shape of the capillary peaks, allowing better quantitation with electronic integration than for the HPLC peaks, where some tailing was observed[17]. The relative standard deviation of the whole procedure (sample preparation and chromatographic analysis) was 2.5%.

The analysis of black pepper extract is shown in Fig. 2B. Compound 3 is caryophyllene, the main constituent of pepper essential oil. The geometrical isomers

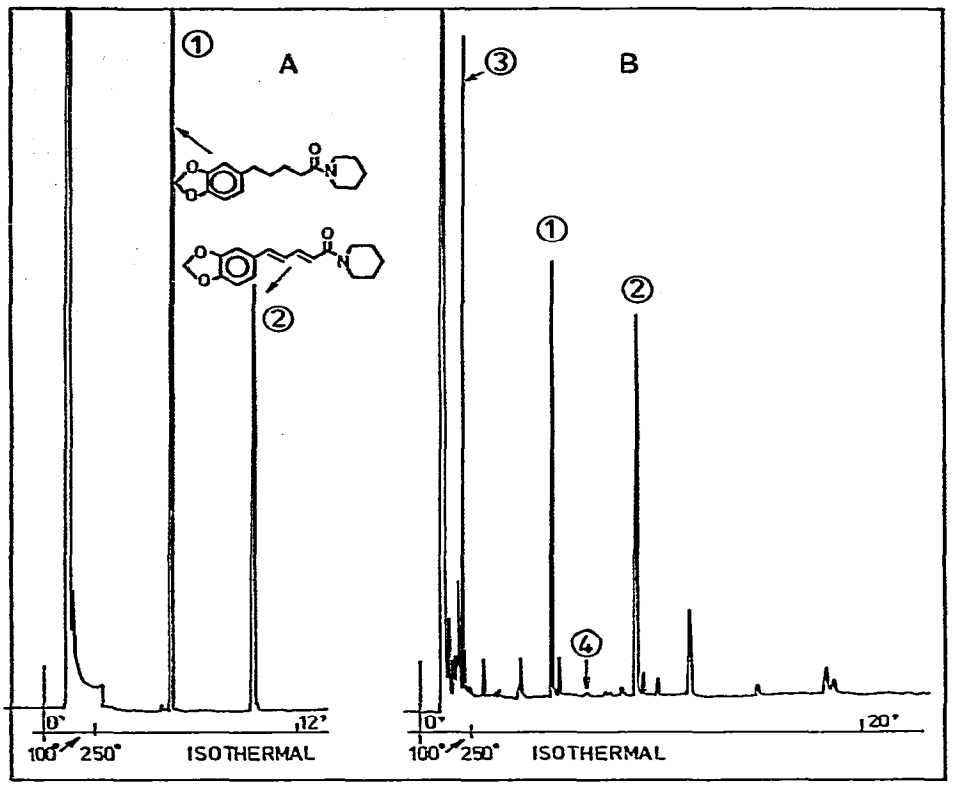


Fig. 2. Glass capillary GC analysis with cold on-column injection of (A) pure piperine and (B) a black pepper extract. Internal standard (peak 1) is tetrahydropiperine; peak 2 is piperine. Column: 25 m × 0.5 mm I.D. HTS-OV-1 column, 250°C isothermal; carrier gas (hydrogen) flow-rate, 4.6 ml/min. For other details and peaks, see text.

of piperine are located in the area marked 4. As can be seen, their presence can be doubted. The identities of the other compounds are still unknown and are the subject of current studies.

TABLE I

COMPARISON OF CGC AND HPLC RESULTS FOR THE DETERMINATION OF PIPERINE IN PEPPERS AND PEPPER EXTRACTS

Pepper or extract	*Piperine content in %*	
	CGC	*HPLC*
South African pepper (1962)	4.4	4.3
Commercial white pepper	3.85	3.6
Commercial black pepper	3.7	3.2
Muntok white pepper	4.0	3.9
Black pepper extract	29.4	27.4
White pepper extract	31.7	29.4

Moving-needle injection applied to quinine–quinidine analysis

Quinine and quinidine are important chemicals requiring rapid analysis in many circumstances, and numerous GC[20–22] and HPLC[23–27] determinations have been reported. The current consensus is that HPLC is to be preferred because packed column GC lacks the necessary efficiency and also requires derivatization of the alkaloids. Improvements to this analysis can be expected with CGC, especially with the recently available high-temperature stationary phases.

Experimental. Samples of the alkaloids in chloroform were prepared. This solvent has to be extracted with sodium hydrogen carbonate solution just before use in order to obtain reproducible results. The sample solutions are stable for several days. Soft drinks and pharmaceutical preparations were made alkaline with 10% sodium hydroxide solution and the alkaloids were extracted with chloroform.

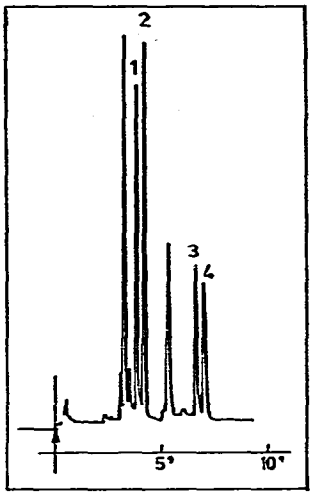
For quantitation piperine proved to be a suitable standard. Piperine is readily available by simple extraction of pepper with methylene chloride. It can easily be purified by recrystallization from dilute methanol or ethanol or from ethyl acetate.

Capillary gas chromatography. The chromatographic behaviour of underivatized alkaloids on different capillary columns was investigated, and it was found that borosilicate or leached soft glass columns were unsuitable. Untreated soft glass (AR-Schott, Jena, G.D.R.) gave acceptable results; some occasional tailing could be removed by sodium chloride dendrite deposition[28]. OV-1, OV-17, OV-225, Superox-4, RSL-702 and RSL-903 were tried as stationary phases (OV phases are produced by Ohio Valley Company and obtainable through most distributors; the last three phases are from RSL-Belgium and are obtainable through RSL, Eke, Belgium or Alltech, Arlington Heights, IL, U.S.A.). All of these stationary phases give good peak shapes. The resolution between quinine and quinidine is zero on OV-1 and improves with increasing polarity of the stationary phase. Resolution is complete only on RSL-903, the most polar stationary phase.

The CGC analyses shown were performed on a 30 m × 0.3 mm I.D. sodium chloride dendrite column coated with a 0.15-μm layer of RSL-903. The samples were injected with the moving-needle injector as described above. A 1-μl volume was introduced on the needle point and the sample was injected isothermally at 280°C. Applying the sample to the needle is much easier than might be expected; breaking off the needle point is to be avoided, and a syringe stop device can be helpful in this respect. A Varian 3700 gas chromatograph equipped with a Varian CDS-111 integrator was used.

Results and discussion. A calibration graph of amount of quinine against piperine was a straight line passing through the origin. The response factor of quinine and quinidine is 1.29 times that of piperine. The relative standard deviation of the points on the graph was between 0.8 and 3%, depending on the peak-height ratio; it was lowest for identical peak heights, as would be expected. Fig. 3 shows a chromatogram of some reference compounds and Fig. 4 the chromatogram of red bark pharmaceutical extract.

This isothermal analysis was applied to the analysis of quinine in soft drinks and of quinine and quinidine in pharmaceutical preparations with good results. Replicate analyses gave standard deviations of 1.97% for quinine in soft drinks and 1.07% and 0.90% for quinine and quinidine, respectively, in pharmaceutical preparations.



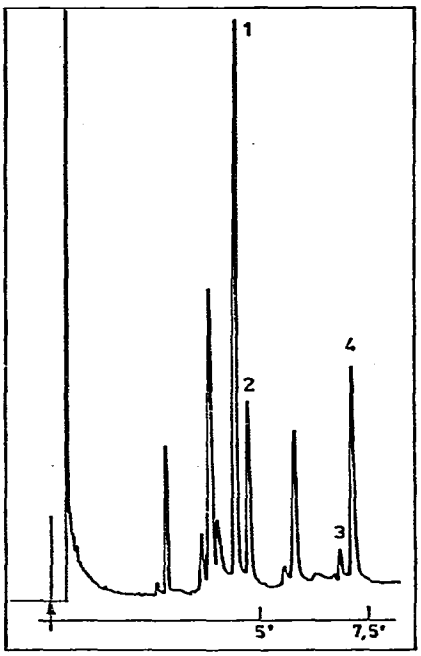


Fig. 3. Glass capillary GC with falling-needle injection of quinine and related alkaloids. Column: 30 m × 0.3 mm I.D. soft glass with sodium chloride dendrite deposition, 280°C isothermal; carrier gas (hydrogen) flow-rate 5 ml/min; chart speed 0.5 cm/min. RSL-903 as stationary phase, static coating, film thickness 0.15 μm. Peaks: 1 = cinchonine; 2 = cinchonidine; 3 = quinidine; 4 = quinine.

Fig. 4. Glass capillary GC with falling-needle injection of a red bark pharmaceutical preparation. Chart speed 1 cm/min. Other conditions as in Fig. 3. Unnumbered peaks so far unidentified. Quantitation is achieved with piperine, eluting shortly after quinine, as internal standard.

CONCLUSION

The "cold on-column" and the "falling-needle" injection techniques are the best methods at present for quantitative CGC. They are applicable to high-temperature CGC, but they have severe limitations. A general-purpose injection technique for CGC is still lacking. If ever developed, this will probably be based on a "gas stream swept out sample holder" (sample loop or gas-purged syringe?). The analysis of piperine and of quinine–quinidine can be effected by either GC or HPLC. Although HPLC is increasingly popular, experience shows us that high-temperature CGC is preferable in this instance in terms of accuracy, reliability and speed.

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