Journal of Chromatography, 348 (1985) 111–122
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 097

SILICA CAPILLARY GAS CHROMATOGRAPHY OF PROSTAGLANDINS WITH ELECTRON-CAPTURE DETECTION AND ITS APPLICATION TO THE FORENSIC INVESTIGATION OF SEXUAL OFFENCES

J. M. F. DOUSE
The Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London SE1 7LP (U.K.)
(Received August 7th, 1985)

SUMMARY

Low picogram levels of the E series prostaglandins, PGE_1, PGE_2, 19-hydroxy PGE_1 and 19-hydroxy PGE_2, in human semen were analysed by silica capillary column gas chromatography with electron-capture detection after conversion to the methyl ester O-trimethylsilyl derivatives of the corresponding B series prostaglandins. The method was used to detect traces of semen on post-coital vaginal swabs, and on rectal, oral and skin swabs after simulated sexual acts. Semen was detectable on a vaginal swab taken 58 h after intercourse, and was readily detectable for at least 6 h on rectal and skin swabs. Preliminary results suggest that the ratios of prostaglandins on vaginal swabs may indicate how recently intercourse occurred.

INTRODUCTION

The majority of sexual offences involve emission of semen and subsequent contamination of the skin, hair, clothing or body of both the victim and the assailant[1]. The identification of seminal traces is thus an important area of forensic science. Methods currently used for detecting such traces include microscopic identification of spermatozoa[2], detection of prostatic acid phosphatase (PAP) activity[3], and enzyme linked immunosorbent assay (ELISA) of the semen protein, p30[4]. These methods vary in specificity and utility.

Microscopic identification of spermatozoa is conclusive evidence of the presence of semen, but spermatozoa may be found in the vagina 5 days after intercourse[2] thus complicating the interpretation of the results in cases involving sexually active women. A further complication arises from the fact that vasectomised men are azoospermic.

The evidential value of PAP activity is limited by the endogenous acid phosphatase activity of the vagina[3] while, with anal and rectal swabs, high acid phosphatase activity is only found when abundant semen is present[5].

The recently reported ELISA method for p30 was found to be more sensitive and specific than the detection of acid phosphatase activity as a "marker" for semen[4]. As with any immunoassay, however, the possibility of a non-specific cross-reaction

0021-9673/85/$03.30

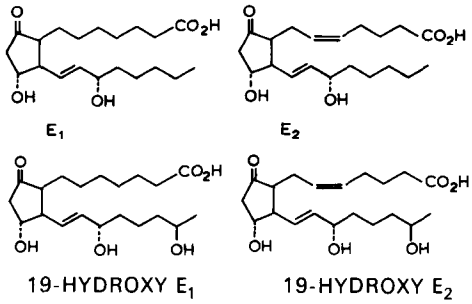

Fig. 1. Major E series prostaglandins in human semen.

cannot be totally excluded in any individual case, and so the evidential value of a positive result is enhanced if the findings can be confirmed by an alternative method.

Five E series prostaglandins, PGE_1, PGE_2, PGE_3, 19-hydroxy-PGE_1 and 19-hydroxy-PGE_2, are present in μg-mg/ml levels in human semen[6] (Fig. 1, Table I). These compounds are thought to originate largely in the seminal vesicles[7] and are present in the ejaculate of vasectomised men in amounts similar to those found in normal semen[7,8]. Various prostaglandins are found in cervical mucus but the levels (ng/ml) are much lower than those of the prostaglandins in semen[9].

The E series prostaglandins are therefore useful markers for the detection of semen traces. Under alkaline conditions, they are readily converted to the corresponding B series prostaglandins which are strongly electron capturing and, after derivatisation, amenable to analysis by capillary column gas chromatography with electron-capture detection[10] (capillary GC–ECD). Such a method offers a useful alternative to the current methods for detecting semen traces.

This paper describes the development of a silica capillary GC–ECD method for the analysis of low picogram levels of the silylated methyl esters of the B series prostaglandins. The samples are prepared for analysis by extraction of the E series prostaglandins from citric acid solution into methyl-*tert.*-butyl ether (MTBE), back extraction into sodium bicarbonate solution, alkaline conversion to the B series prostaglandins, acidification and extraction of the B series prostaglandins into MTBE followed by esterification with diazomethane and silylation with N,O-bis(trimethylsilyl)acetamide (Fig. 2). The prostaglandins were recovered in good yield by this procedure with low background levels of coextractives.

TABLE I

MAJOR E SERIES PROSTAGLANDIN LEVELS FOUND IN HUMAN SEMEN

	Concentration in semen (μg/ml)*	
	Mean	*Range*
PGE_1 and PGE_2	73.2	2–272
PGE_3	5.5	3.5–7.8
19-Hydroxy PGE_1 and 19-hydroxy PGE_2	267.0	53–1094

* Data from ref. 6.

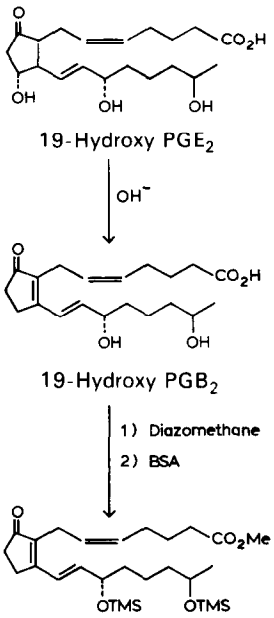

Fig. 2. Conversion of E series prostaglandins to B series methyl ester O-trimethylsilyl derivatives, with 19-hydroxy PGE_2 as an example.

The method was used to detect semen in various matrices of forensic interest with excellent selectivity and sensitivity.

EXPERIMENTAL

Reagents

Prostaglandins, PGB_1 and PGB_2, were obtained from Sigma (Poole, U.K.). N,O-bis(trimethylsilyl)acetamide (BSA) was supplied by Pierce (Chester, U.K.). Citric acid was Analar grade (B.D.H., Poole, U.K.). Sodium bicarbonate and anhydrous sodium sulphate were Laboratory grade (May and Baker, Dagenham, U.K.). Sodium hydroxide and hydrochloric acid were G.P.R. grade (B.D.H.). All solvents were pesticide grade (Fisons, Loughborough, U.K.) except methyl-*tert.*-butyl ether (MTBE) which was HPLC grade (Rathburn, Walkerburn, U.K.).

Anhydrous sodium sulphate was cleaned by repeated vortex extraction with MTBE followed by evaporation under a stream of nitrogen at 75°C.

All glassware was silanized as described previously[11] and thoroughly rinsed with MTBE before use.

Diazomethane was prepared[12] as a solution in diethyl ether.

Stock solutions of prostaglandins

Solutions of prostaglandin standards in MTBE were stored in unsilanized glass in darkness at 4°C.

Collection and storage of semen samples

Semen obtained by masturbation was donated by volunteers. Samples were collected in unsilanized 1-oz. glass screw cap vials (Aimer Products, London, U.K.) and stored at −20°C.

Sampling procedures and storage of swabs

Dry, plain, sterile cotton wool swabs (Medical Wire and Equipment, Corsham, U.K.) were used to collect samples after intercourse or simulated sexual assault. The swabs were moistened with distilled water before sampling dry semen on skin. Swabs that were not analysed immediately were stored at −20°C. Swabs provided for a blind trial were air dried before storage at −20°C.

Pre- and post-coital vaginal swabs were provided by volunteer couples from the scientific staff of this laboratory.

Anal intercourse was simulated by introducing 1 ml of fresh semen 10 cm into the rectum of a volunteer using a vaseline lubricated 1 ml syringe (10 cm × 0.8 cm O.D., Luer Sabre, Gillette Surgical, London, U.K.). Rectal swabs to a depth of about 12 cm were taken before and 7 h after the introduction of semen.

Oral intercourse was simulated by a volunteer who swirled 1–2 ml of his own fresh ejaculate around his mouth for several seconds before expelling it. Oral swabs were taken before and immediately afterwards.

A dried semen stain on skin was prepared by allowing semen to dry naturally on exposed skin. Swabs were taken before and 6 h afterwards.

Extraction of semen

Semen (0.5 ml), aqueous citric acid solution (0.5 ml, 200 g/l) and MTBE (1.5 ml) were placed in a Quickfit ground glass stoppered centrifuge tube and rotated for 15 min. The mixture was then centrifuged (2 min, 1700 *g*) and the MTBE layer was transferred to a conical centrifuge tube.

Extraction of swabs

Swabs were rinsed with citric acid (0.5 ml, 200 g/l) in a conical centrifuge tube by repeated agitation followed by expression of absorbed liquid on the upper dry surface of the tube. The resulting aqueous mixture was vortex extracted with MTBE (1.5 ml) for 30 s and centrifuged (2 min, 1700 *g*). The MTBE layer was then transferred to a conical centrifuge tube. The extraction was repeated and the MTBE extracts were combined.

Sodium bicarbonate back extraction of MTBE extracts

Aqueous sodium bicarbonate (0.5 ml, 16 g/l) was added to the MTBE extract of a semen sample or swab. The mixture was vortexed for 1 min and centrifuged (2 min, 2500 rpm). The MTBE layer was removed and extracted a second time with bicarbonate in a fresh conical tube. The combined bicarbonate extracts were washed by vortexing for 10 s with MTBE (1.5 ml) which was discarded.

Alkaline conversion of E series prostaglandins to B series prostaglandins

Sodium hydroxide (0.5 ml, 1 *M*) was added to the bicarbonate extract. The mixture was briefly vortexed and incubated for 10 min at 75°C for the conversion to occur.

Extraction of B series prostaglandins

The alkaline solution containing the B series prostaglandins was acidified with hydrochloric acid (0.5 ml, 6 *M*) and vortex extracted with MTBE (1.5 ml) for 30 seconds. The mixture was centrifuged (2 min, 1700 *g*) and the MTBE layer was transferred to a clean conical centrifuge tube. The extraction was repeated and the combined MTBE extracts were dried by brief vortex mixing with anhydrous sodium sulphate (approx. 100 mg). The mixture was centrifuged (2 min, 1700 *g*) and the MTBE layer was transferred to a screw cap vial. The sodium sulphate was washed with MTBE (1 ml) which was added to the dried MTBE extract.

Derivatisation

Methylation. Ethereal diazomethane (50 μl) was added to the dried MTBE extract and the faintly yellow solution was allowed to stand for 5 min. The solution was then evaporated to dryness under nitrogen at room temperature.

Silylation. BSA (100 μl, 20% in acetone) was added to the methylated residue. The vial was capped and incubated at 75°C for 10 min. Excess reagent was removed by evaporating to dryness under nitrogen at 75°C. The residue was immediately dissolved in cyclohexane (50–2000 μl) and 0.5 or 1 μl was analysed by capillary GC–ECD.

Silica capillary GC–ECD

The gas chromatograph (Carlo Erba Model 4160) was equipped with a Model HT-25 ^{63}Ni electron-capture detector and a Model 251 detector control module. The Grob split–splitless injector was fitted with a 0.25-ml silica injection port liner and used in the splitless mode with no septum purge or purge of the injection port after injection.

Two flexible fused-silica capillary columns (0.25 mm I.D.) were used (SGE, Milton Keynes, U.K.). Column 1 was 11 m long and 4 months old. Column 2 was 12,5 m long and new. Both columns were coated with a 0.25-μm film thickness of BP-1 stationary phase.

The following conditions were used. Carrier gas: helium (Air products, Southampton, U.K.); flow-rate 7 ml/min (25°C). Injection port pressure: 50 p.s.i. Make-up gas: 5% methane in argon (Air Products, Southampton, U.K.); flow-rate 43 ml/min (25°C), pressure 2.1 kg/cm^2. Injection port temperature: 250°C. Detector temperature: 300°C. Temperature programme: 150°C programmed at 39.9°/min to 320°C; held at 320°C for 3 min; extra cool down time, 3 min. Conditioning time: 1 min.

The electron-capture detector was operated in the constant current mode with a potential of 50 V, a 1-μs pulse width and a standing current of 0.85 nA.

Other details of the capillary GC–ECD analysis have been described previously[13].

Silica capillary GC with flame ionisation detection

The gas chromatograph (Carlo Erba Model 4300) was equipped with a Model 40 flame ionisation detector and a Model 480 electrometer. The Grob split–splitless injector was operated in the split mode with a split ratio of 10:1. A flexible fused-silica capillary column (25 m × 0.25 mm I.D.) coated with a 0.25-μm film of BP-1 was used (SGE).

The following conditions were used. Carrier gas: helium; flow-rate, 6.0 ml/min (25°C). Injection port pressure: 3.4 kg/cm^2. Injection port temperature: 250°C. Detector temperature: 300°C. Temperature programme: 140°C held for 1 min then programmed at 39.9°/min to 330°C; held at 330°C for 4 min; extra cool down time, 3 min. Conditioning time: 1 min.

Gas chromatography–mass spectrometry (GC–MS)

The gas chromatograph (Carlo Erba Model 4160) was linked via a direct insertion interface to a 12-12F quadrupole mass spectrometer (VG Analytical, Altrincham, U.K.). The Grob split–splitless injector was used in the splitless mode with no septum purge and with automatic purging of the injection port with helium 30 s after injection. A flexible fused-silica capillary column (12.5 m × 0.2 mm I.D.) coated with a 0.25-μm film of BP-1 was used (SGE).

The following conditions were used. Carrier gas: helium; vacuum pressure at

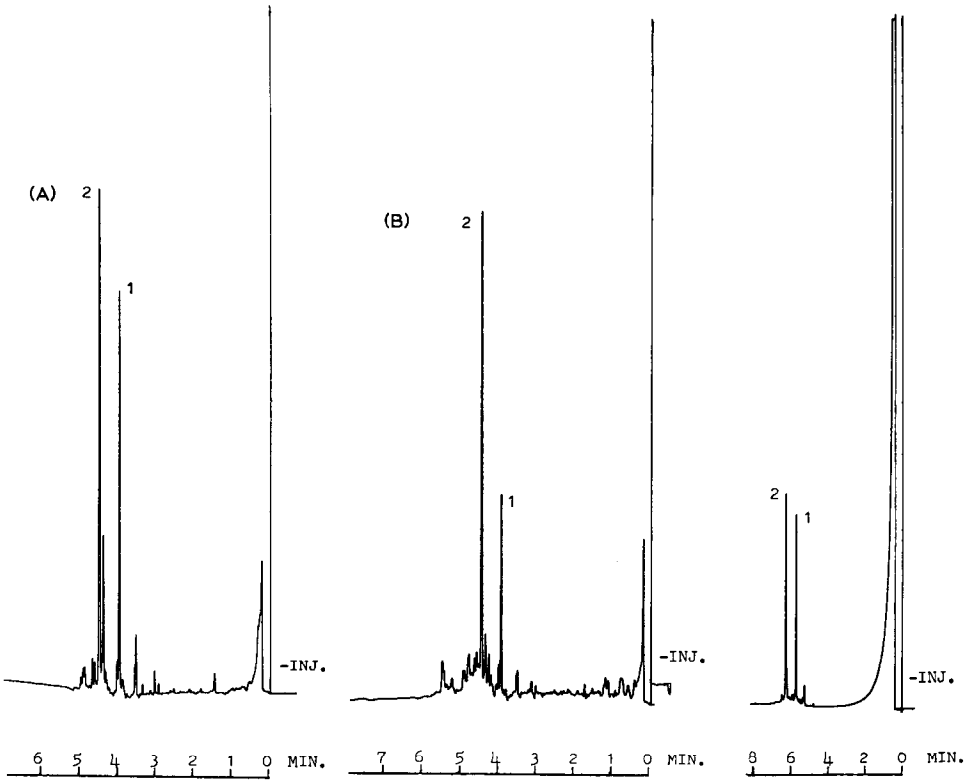

Fig. 3. Analysis of human semen extracts by capillary GC–ECD showing methyl ester O-TMS derivatives of B prostaglandins (1) and 19-hydroxy B prostaglandins (2). (A) Extract of 0.5 ml normal semen; 0.007% of extract injected; column 1; attenuation × 64. (B) Extract of 0.1 ml semen from a vasectomised subject; 0.17% of extract injected; column 1; attenuation × 128.

Fig. 4. Capillary GC–FID analysis of an extract of 2 ml normal human semen showing methyl ester O-TMS derivatives of B prostaglandins (1) and 19-hydroxy B prostaglandins (2). 1% of extract injected; attenuation × 8.

end of column, 10^{-5} Torr (oven temp. 260°C). Injection port temperature: 250°C. Injection port pressure: 0.7 kg/cm^2. Interface temperature: 250°C. Temperature programme: 100°C held for 1 min then programmed at 30°/min to 280°C; held at 280°C for 6 min; extra cool down time, 2 min.

The mass spectrometer was operated in the electron-impact mode with an electron energy of 70 eV. The source temperature was 200°C.

RESULTS AND DISCUSSION

The object of this work was to determine whether capillary GC–ECD analysis of prostaglandins is a feasible method of detecting semen traces in sexual offence cases. A working method was devised and applied to a range of samples with very encouraging results. Further work is required to optimise the parameters of the analysis and an extensive background survey is necessary before the method can be applied to forensic casework.

The sample preparation was designed to afford good recovery of the analytes with minimal interference from endogenous co-extractives. Evaporation of the de-

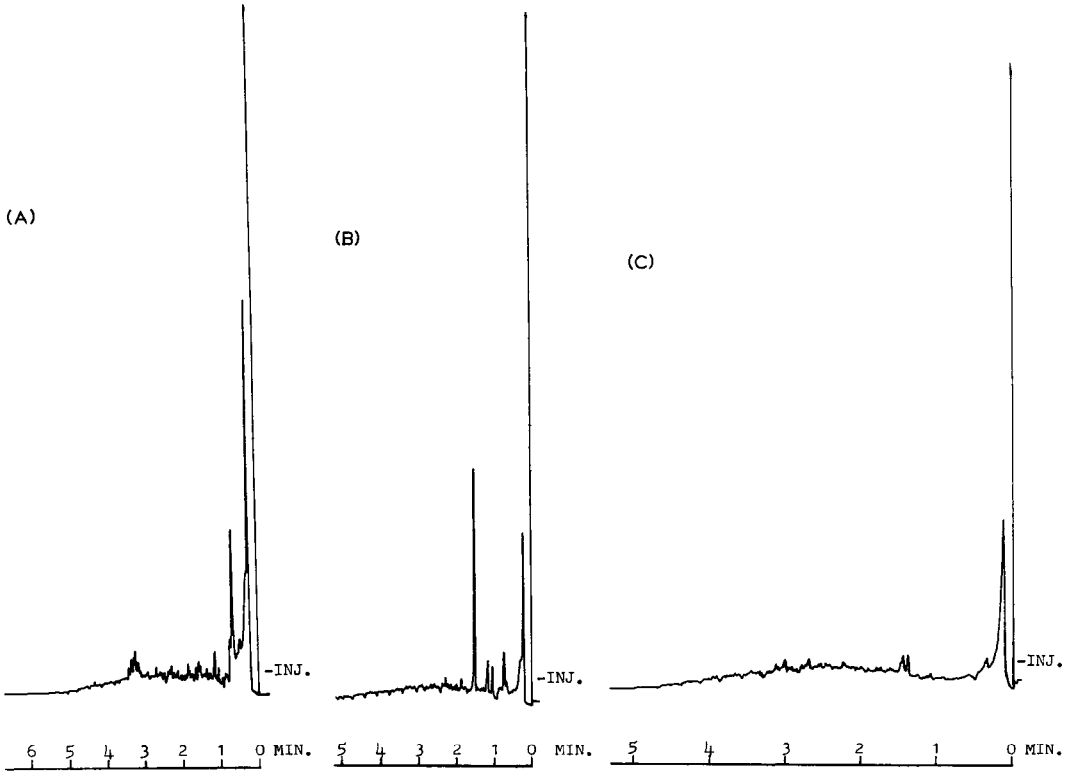

Fig. 5. Capillary GC–ECD analysis of vaginal swab extracts taken at least 7 days after intercourse. (A and B) Non-menstrual swabs from 2 different donors; 0.5% of extract injected; column 1; attenuation ×128. (C) Swab from a third donor whose menstrual period was almost complete (blood was present on the swab); 1% of extract injected; column 2; attenuation ×64.

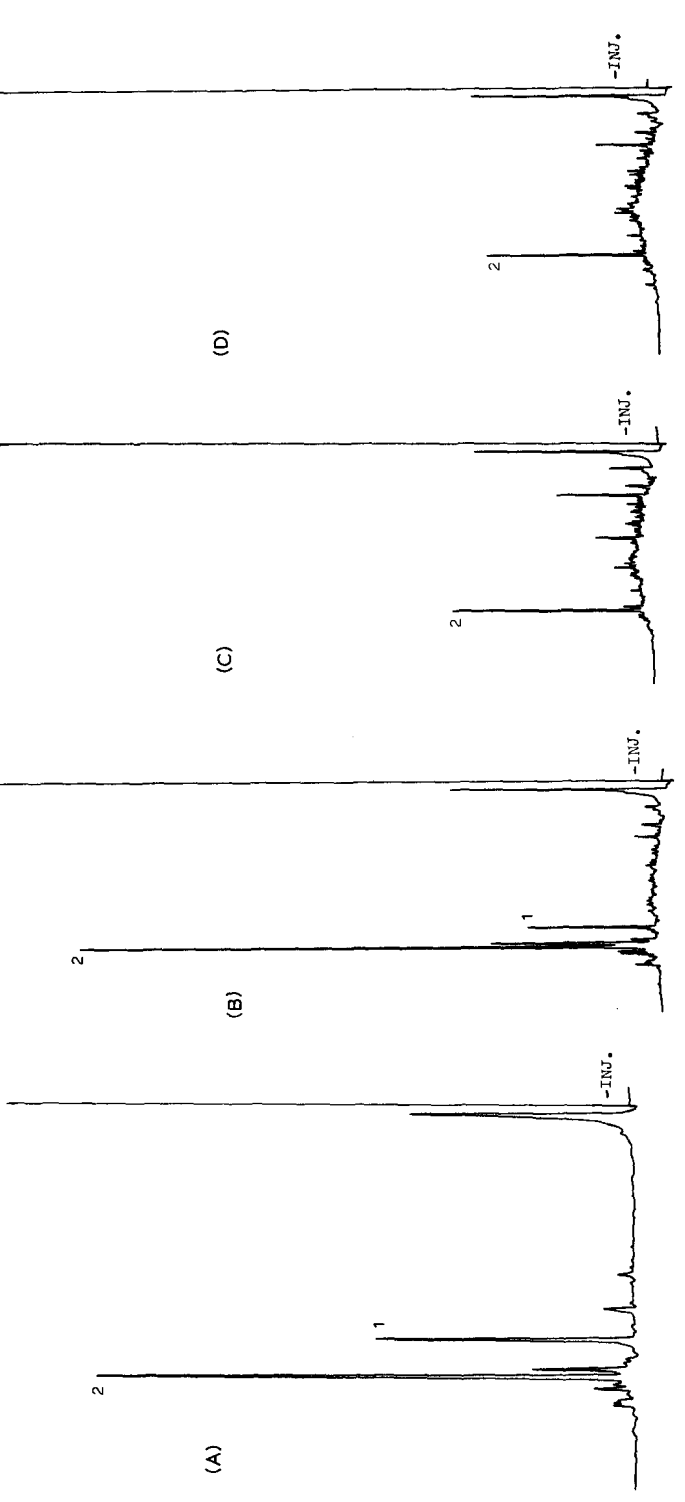

Fig. 6. Capillary GC–ECD analysis of post-coital vaginal swab extracts taken at various times from 4 different donors showing methyl ester O-TMS derivatives of B prostaglandins (1) and 19-hydroxy B prostaglandins (2). (A) Donor 1; 0 h. (B) Donor 2; 3.25 h. (C) Donor 3; 10 h. (D) Donor 4; 12 h. 0.06% of extract A was injected; column 2; attenuation ×64. 0.5% of extracts B, C and D were injected; column 1, attenuation ×128.

rivatised analytes under nitrogen for 10 min at 75°C to remove excess BSA also removed the more volatile co-extractives. Further optimisation of the procedure may increase the recovery of analytes and significantly reduce the chromatographic background, enabling even smaller amounts of prostaglandins to be analysed.

Pure samples of the E series prostaglandins or the 19-hydroxy B series prostaglandins were not available and so GC–MS was used to identify the major peaks in the chromatograms.

The capillary GC–ECD profile of semen extracts from normal or vasectomised subjects contained two major and a number of minor peaks (Fig. 3). Analysis by capillary GC-FID (Fig. 4) or GC–MS in the total ion current mode confirmed that there were only two major peaks in the chromatogram. The shorter-retained major peak had a retention identical to that of the methyl ester O-TMS derivatives of PGB_1 and PGB_2 which were not resolved under the conditions used. GC–MS confirmed that the shorter retained major peak contained the PGB_1 and PGB_2 derivatives since the mass spectrum contained the expected molecular ions of *m/e* 422 and 420 along with characteristic fragment ions of *m/e* 351, 349, 323 and 321. The mass spectrum of the longer retained major peak contained the molecular ions, *m/e* 510 and 508, of

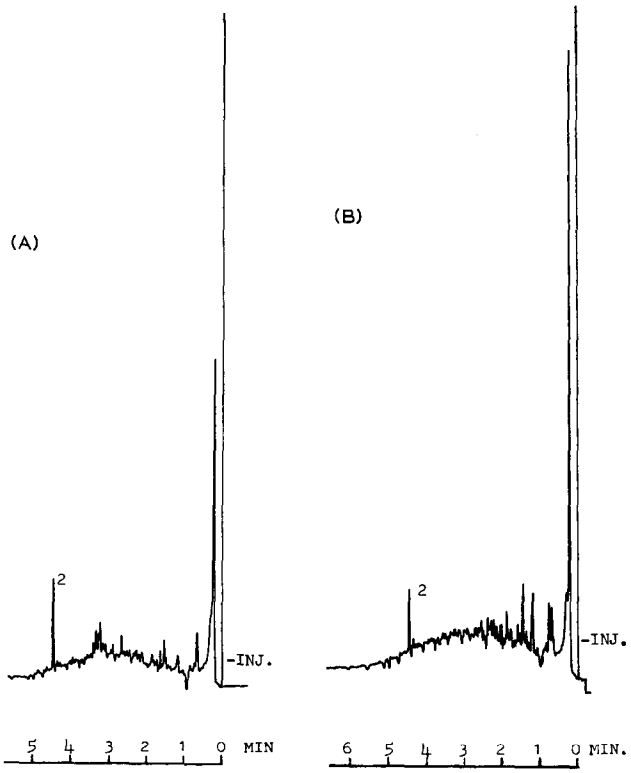

Fig. 7. Capillary GC–ECD analysis of post-coital vaginal swab extracts from 2 different donors showing methyl ester O-TMS derivatives of 19-hydroxy B prostaglandins (2). (A) Swab taken 50 h after intercourse; 0.5% of extract injected; column 1; attenuation ×128. (B) Swab taken 58 h after intercourse; 0.75% of extract injected; column 1; attenuation ×64.

the methyl ester O-TMS derivatives of 19-hydroxy PGB_1 and 19-hydroxy PGB_2 as well as the characteristic ions of *m/e* 351, 349, 323 and 321. It is reasonable to conclude, therefore, that the two major peaks in the chromatograms are derived from the major semen prostaglandins, PGE_1, PGE_2, 19-hydroxy PGE_1 and 19-hydroxy PGE_2.

Vaginal swabs from three subjects who had abstained from intercourse for at least 7 days contained none of the major prostaglandins found in semen (Fig. 5). Menstrual blood was present on one swab.

A vaginal swab taken immediately after intercourse (Fig. 6A) showed the profile of major and minor peaks found in a control semen extract from the male partner (Fig. 3A). Over a period of hours, the minor peaks and the shorter-retained major peak decreased until, after 10 h, only the longer-retained major peak containing the 19-hydroxy prostaglandins could be detected (Fig. 6B, C and D). Similar changes with time were found on swabs from 4 different subjects.

In a blind trial, 2 pre-coital vaginal swabs were easily distinguished from 6 post-coital swabs, semen traces on swabs from 2 different subjects being detected 50 and 58 h after intercourse respectively (Fig. 7).

19-Hydroxy prostaglandins were detected on a rectal swab 7 h after simulated anal intercourse; a rectal swab taken before the experiment showed no trace of E or B series prostaglandins (Fig. 8).

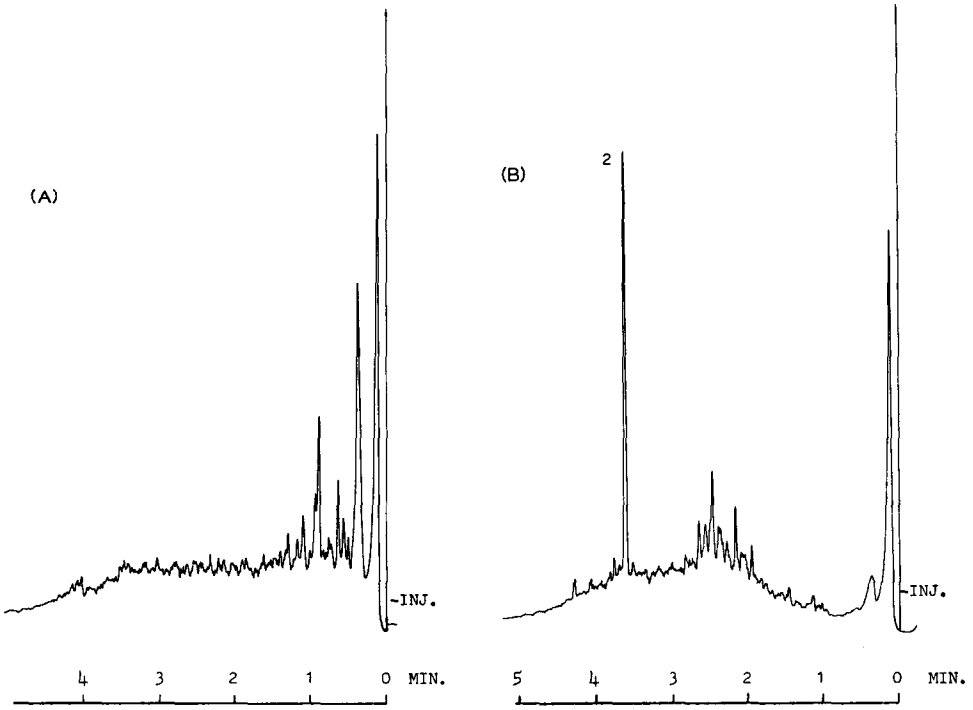

Fig. 8. Capillary GC–ECD analysis of rectal swab extracts. (A) Before and (B) 7 h after simulated anal intercourse showing methyl ester O-TMS derivatives of 19-hydroxy B prostaglandins (2). 1% of each extract was injected; column 2; attenuation ×64.

A swab taken from a dried semen stain on skin after 6 h contained the parent prostaglandins and their 19-hydroxy derivatives; a control skin swab was blank (Fig. 9).

The parent prostaglandins and their 19-hydroxy derivatives were found on an oral swab immediately after simulated oral intercourse; a control oral swab was blank (Fig. 10).

The results demonstrate the potential of the method in the investigation of sexual offences. Semen can be detected on a vaginal swab at least 58 h after intercourse. Optimisation of the experimental procedure should increase the sensitivity of the analysis and reduce the chromatographic background, thus considerably extending the post-coital interval during which the method can be used.

The specificity of the method is high since the analytes are small molecules of known structure whose identities can be confirmed by GC–MS.

The results indicate that the parent prostaglandin levels decline rapidly in the first few hours after intercourse leaving only the 19-hydroxy derivatives. It may therefore be possible to estimate the time since intercourse from measurements of prostaglandin ratios and thus determine whether semen is present as a result of recent intercourse or whether traces have persisted from previous coitus. In this context, the use of a more polar chromatographic stationary phase to resolve the co-eluting prostaglandins may provide valuable additional information. Further work is in progress.

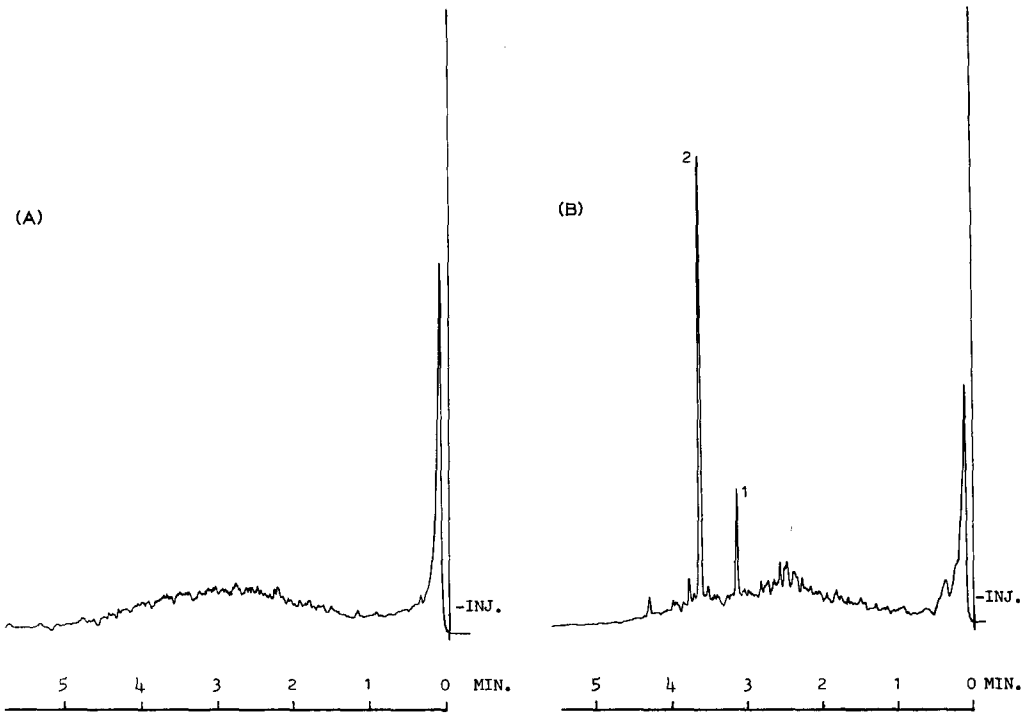

Fig. 9. Capillary GC–ECD analysis of skin swab extracts. (A) Swab of blank skin. (B) Swab of a 6 h old dried semen stain on skin showing methyl ester O-TMS derivatives of B prostaglandins (1) and 19-hydroxy B prostaglandins (2). 1% of each extract was injected; column 2; attenuation ×64.

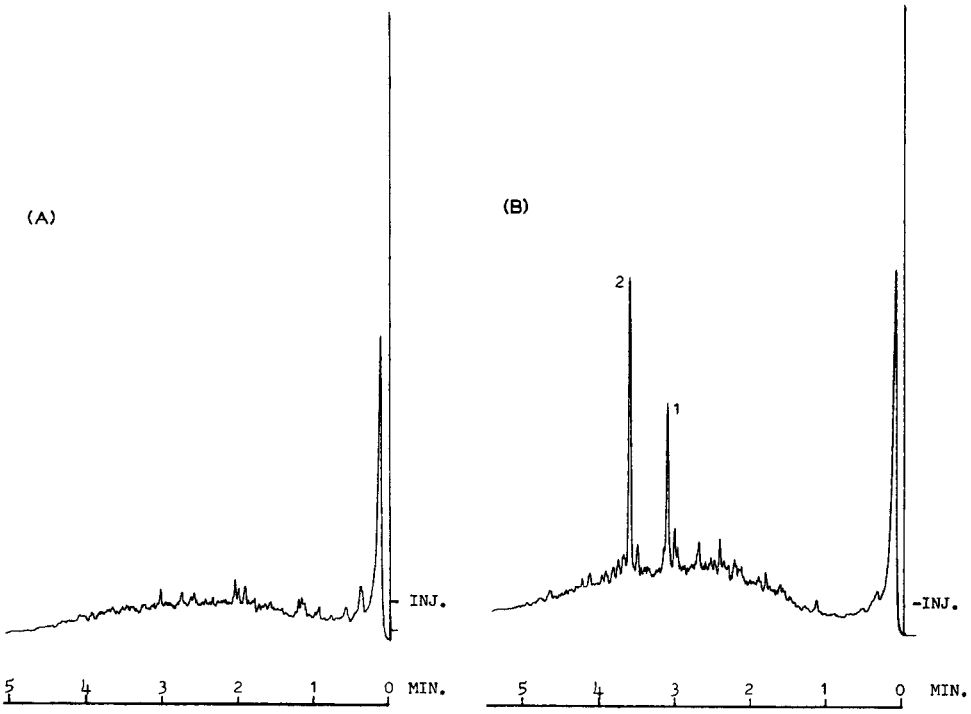

Fig. 10. Capillary GC–ECD analysis of oral swab extracts. (A) Before and (B) immediately after simulated oral intercourse showing methyl ester O-TMS derivatives of B prostaglandins (1) and 19-hydroxy B prostaglandins (2). 0.5% and 1% respectively of extracts A and B were injected; column 2; attenuation ×64.

ACKNOWLEDGEMENTS

I am grateful to all those who contributed samples, to Sheila Wotherspoon for organising the collection of swabs for the blind trial, to P. Burke and M. J. Whitehouse for the mass spectral data, and to B. B. Wheals and R. N. Smith for helpful advice.

REFERENCES

1 S. H. Burges, *The New Police Surgeon,* Hutchinson Benham, London, 1978, p. 222.
2 G. M. Willott and J. E. Allard, *Forensic Sci. Int.*, 19 (1982) 135.
3 G. M. Willot, *J. Forensic Sci. Soc.*, 12 (1972) 363.
4 H. C. B. Graves, G. F. Sensabaugh and E. T. Blake, *N. Engl. J. Med.*, 312 (1985) 338.
5 A. Davies, *Med. Sci. Law*, 18 (1978) 174.
6 T. Mann and C. Lutwak-Mann, *Male Reproductive Function and Semen,* Springer Verlag, New York, 1981, p. 315 and references cited therein.
7 K. Gerozissis, P. Jouannet, J. C. Soufir and F. Dray, *J. Reprod. Fert.*, 65 (1982) 401.
8 K. C. Srivastava, R. K. Bansal and K. P. Tiwari, *Dan. Med. Bull.*, 28 (1981) 201.
9 B. Charbonnel, M. Kremer, K. Gerozissis and F. Dray, *Fert. Steril.*, 38 (1982) 109.
10 M. Korteweg, G. Verdonk, P. Sandra and M. Verzele, *Prostaglandins*, 13 (1977) 1221.
11 J. M. F. Douse, *J. Chromatogr.*, 234 (1982) 415.
12 L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis,* Wiley, New York, 1967, p. 192.
13 J. M. F. Douse, *J. Chromatogr.*, 301 (1984) 137.