*Journal of Chromatography*, 623 (1992) 191–195
Elsevier Science Publishers B V , Amsterdam

CHROM 24 456

# Short Communication

# Improved determination of brofaromine by capillary gas chromatography and by utilization of a multi-purpose injector

P H Degen, F Risser and J W Lauber

*Pharma Research and Development, Pharmaceuticals Division, Ciba-Geigy Ltd , Basle (Switzerland)*

(First received January 31st, 1992, revised manuscript received June 30th, 1992)

ABSTRACT

A significantly improved method for the determination of the monoamine oxidase A inhibitor brofaromine hydrochloride and its major metabolite is described A newly constructed multi-purpose injector for capillary column gas chromatography (cGC) was utilised The injector can be used either in split/splitless or hot or cold quasi-on-column mode For this purpose it was used in the hot on-column mode The injector is equipped with a septum cooling device, allowing injection temperatures of up to 400°C without septum glueing The utilization of cGC with the new highly thermally stable injector showed improvements over the standard GC procedure in terms of retention time stability and ease of operation

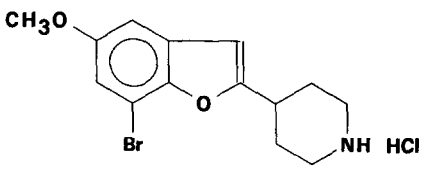
## INTRODUCTION

A procedure for the determination of brofaromine, a monoamine oxidase A inhibitor (MAOI), and its major metabolite, O-desmethylbrofaromine (Fig 1), by packed-column gas chromatography (GC) was reported in a previous paper [1] The method is based on liquid–liquid extraction followed by acylation with heptafluorobutyric anhydride (HFBA) and GC using an OV-17 column Using packed columns, the chromatography of the major metabolite required time-consuming conditioning, to overcome adsorption losses, by repeated injections of O-desmethylbrofaromine The utilization of capillary GC (cGC) columns in combination with automated quasi-on-column injection (injection into a precolumn or retention gap) was achieved by the use of a newly designed multi-purpose injector, which is based on the Grob injector [2]

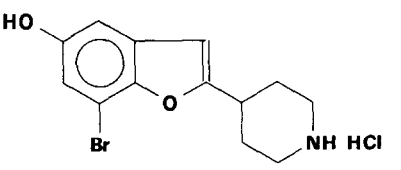
The technique of hot on-column injections has been discussed before but has not often been utilized owing to a lack of adequate hardware [3,4] The advantages of this injector are its flexibility in its use as a hot or cold quasi-on-column or as a split/splitless injector and especially its homogeneous temperature field over the entire injector To avoid cold parts or spots at the splitter outlet and septum purge, these tubings are integrated within the heated block up to the exit valves In addition, the dead volumes of these tubings are minimized The problems arising from temperature gradients in the injector have been recognized and discussed in detail by Grob [2] The temperatures of the top part

*Correspondence to* Dr P H Degen, Pharma Research and Development, Pharmaceuticals Division, Ciba-Geigy Ltd , Basle, Switzerland

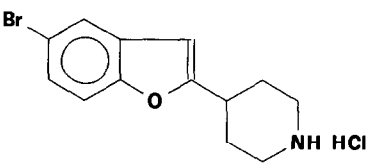
0021-9673/92/$05 00 




Brofaromine hydrochloride




O-desmethyl brofaromine




Internal standard

Fig 1 Structures of brofaromine hydrochloride, O-desmethylbrofaromine hydrochloride and the internal standard

of commercial injectors are kept relatively low in order to preserve the heat-sensitive septum It was demonstrated that normalized peak areas of *n*-alkanes ($C_{10}$–$C_{44}$) showed the best stability when the top part of the injector was also actively heated When injector temperatures above 250°C are to be used, an additional intensive septum cooling system is recommended to avoid septum glueing The septum cooling system allows the utilization of injector temperatures as high as 400°C

## EXPERIMENTAL

### *Method*

The extraction and derivatisation procedure was used as described previously [1] Briefly, brofaromine [4-(7-bromo-5-methoxy-2-benzofuranyl)piperidine hydrochloride, $C_{14}H_{16}BrNO_2$ HCl, mol wt 346 65], its O-desmethyl metabolite [4-(7-bromo-5-hydroxy-2-benzofuranyl)piperidine hydrochloride, $C_{13}H_{14}BrNO_2$ HCl, mol wt 332 63] and the internal standard [4-(5-bromo-2-benzofuranyl)piperidine hydrochloride, $C_{13}H_{14}BrNO$ HCl, mol wt 316 63] are extracted with diethyl ether–dichloromethane (4 1, v/v) at basic pH After evaporation of the solvents, the compounds are acylated with HFBA and chromatographed

### *Gas chromatographic instrumentation*

The GC instrument used was a Carlo Erba MEGA Series Model 5360, equipped with a Model 91 Ciba-Geigy injector (Mechanical Workshop, Ciba-Geigy, Basle, Switzerland) and a A200S autosampler (Carlo Erba, Milan, Italy, for Europe and Leap Technology, Chapel Hill, NC, USA, for the USA) Data evaluation was performed with a Merck–Hitachi D-2500 MEGA integrator

The Ciba-Geigy injector, tooled from stainless steel, can be operated either in the split/splitless or in the cold or hot quasi-on-column injection mode All three injection techniques can be automated with the A200S autosampler For application with brofaromine, the injector was used in the hot quasi-on-column mode with the splitter open, splitting ratio 1 20, during the entire analysis Using hot on-column injection, the peak shape, the limit of detection and the quantitative reproducibility of the chromatograms were optimal

Sectional views of the injector in the three modes are shown in Fig 2 The diagrams show the possible

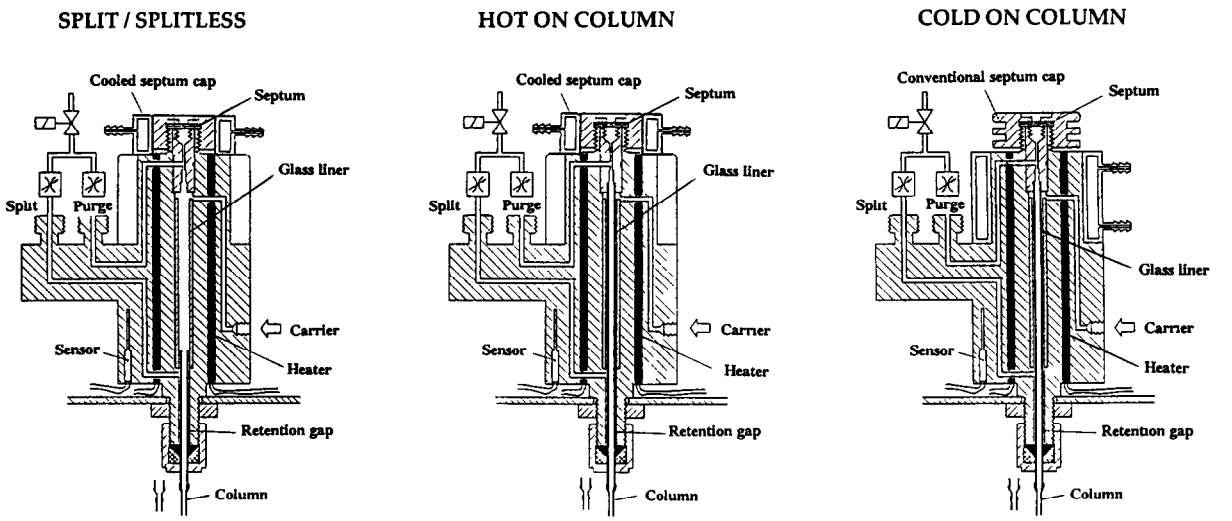



Fig 2 Sectional views of the Ciba-Geigy Model 91 detector Split/splitless mode and hot on-column mode septum cooling device installed Cold on-column mode conventional septum cap installed

different configurations of the injector, *e g* , if the injector is to be used for cold on-column, injections exclusively, no septum cooling cap would be necessary For the split/splitless mode, the column ending is positioned just above the splitter outlet connection For both other modes, the column ending is positioned at the very top of the injector below the septum purge exit

Advantages of this injector over existing models are the temperature stability over the entire length of the injector, the placement of all gas lines within the uniformely heated injector body and its versatility In addition, it can be fitted to most commercial gas chromatographs

*Column*

GC analyses were performed with a 50 m × 0 3 mm I D glass capillary column [Duran-50 glass tubing obtained from Schott Ruhrglas, Bayreuth, Germany, drawn on a GCDM Model 03 instrument (Brechbuhler, Schlieren, Switzerland)] The capillaries were pretreated, coated and immobilized with SE-54, chemically bonded and cross-linked as described by Grob [5]

For autosampler injections an additional 18 cm × 0 5 mm I D glass tube was mounted at the start of the column This was connected in front of the analytical column with a 2-cm length of fused silica (without polyimide) between the additional retention gap and the column, to prevent collapse during the connection process The dimensions of the glass tubing allow the use of commercially available microsyringes (0 5 mm O D needle, *e g* , Hamilton 701N)

The column was used with 3-m retention gaps at both ends With such columns, the direction of the gas flow is not critical, *i e* , the column may be installed either way The loss of resolution due to the retention gap at the end of the column was insignificant for this application The carrier gas was hydrogen with a precolumn pressure of 100 kPa

*Conditions*

The injector temperature was 250°C and the detector temperature was 250°C The column oven was heated using a temperature programme as follows 120–240°C at 15°C/min, 240–290°C at 2 5°C/min, final temperature 290°C maintained for 6 min before a new cycle The retention times of the derivatives under these conditions were as follows O-desmethyl metabolite, 16 03 min, brofaromine, 18 05 min, and internal standard, 19 60 min

## RESULTS AND DISCUSSION

### *Calibration*

Calibration graphs were prepared by analysis of plasma samples spiked with brofaromine hydrochloride and O-desmethylbrofaromine hydrochloride (metabolite) The peak-height ratios (brofaromine and metabolite/internal standard) were then plotted against original concentrations and analysed by quadratic least-squares regression analysis ($y = a + bx + cx^2$) The parameters obtained are given in Table I

Chromatograms of blank matrix extracts and extracts of plasma samples spiked with brofaromine, metabolite and internal standard are given in Fig 3

### *Method validation*

The method was validated as follows Blank human plasma samples were spiked with brofaromine hydrochloride (14–588 nmol/l) and with O-desmethylbrofaromine hydrochloride (10–270 nmol/l) and analysed as described The chromatograms were evaluated and the given − found results were subjected to a linear least-squares regression analysis ($y = a + bx$) The parameters obtained are given in Table II

TABLE I

CALIBRATION CURVES

| Parameter[a] | Brofaromine | Metabolite |
|---|---|---|
| Range | 14–588 pmol | 15–302 pmol |
| *a* | 0 0036 | 0 0065 |
| *b* | 0 0030 | 0 0040 |
| *c* | −0 0000009 | −0 0000002 |
| *R* | 0 9999 | 0 9997 |
| $S_y$ | 0 0101 | 0 0092 |

[a] $R$ = Correlation coefficient, $S_y$ = estimated standard deviation

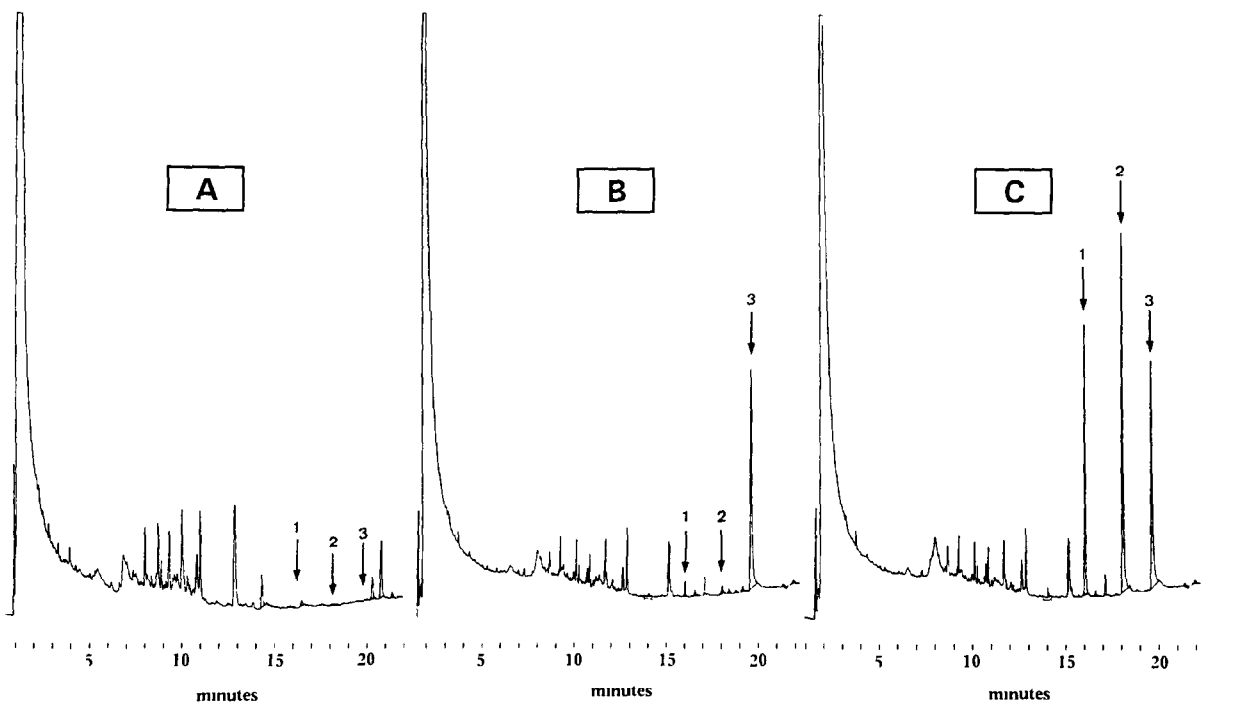



Fig 3 Chromatograms of plasma extracts (A) blank human plasma (1 ml), (B) human plasma (1 ml) spiked with brofaromine HCl (14 nmol/l), O-desmethyl metabolite (15 nmol/l) and internal standard (1 26 μmol/l), (C) human plasma (1 ml) spiked with brofaromine HCl (577 nmol/l), O-desmethyl metabolite (300 nmol/l) and internal standard (1 26 μmol/l) 1 = O-Desmethyl metabolite, 2 = brofaromine, 3 = internal standard

TABLE II

METHOD VALIDATION

| Parameter[a] | Brofaromine | Metabolite |
|---|---|---|
| Range | 14–588 nmol/l | 10–270 nmol/l |
| $a$ | 3 8088 | −0 4094 |
| $b$ | 1 0136 | 1 0261 |
| $R$ | 0 9986 | 0 9953 |
| $S_y$ | 10 3289 | 9 7682 |

[a] $R$ = Correlation coefficient, $S_y$ = estimated standard deviation

*Reproducibility of the retention times*

In a pharmacokinetic study, extracts of 58 different plasma samples (varying concentrations over the entire range) were injected into the same column over a period of 3 weeks The relative standard deviations were 0 05, 0 07 and 0 07% for O-desmethyl metabolite, brofaromine and the internal standard, respectively This high reproducibility of <0 1% was probably achieved owing to the high temperature stability of the injector With conventional split/splitless injectors the reproducibilities of retention times and the determinations were unsatisfactory

## CONCLUSIONS

The utilization of cGC and a newly constructed multi-purpose injector resulted in a dramatic improvement in the determination of brofaromine and its major metabolite in plasma The injector has a high temperature stability, resulting in excellent reproducibility of the chromatograms In addition, the injector may easily be used for other types of cGC injection techniques and can also be fitted to most commercial gas chromatographs

## ACKNOWLEDGEMENT

Thanks are due to Mr H P Ruedisuehli for the skilful manufacture of the split/splitless, quasi-on-column injector

## REFERENCES

1 W Schneider, B Keller and P H Degen, *J Chromatogr*, 488 (1989) 275–282
2 K Grob, *Classical Split and Splitless Injection in Capillary GC*, Huthig, Heidelberg, 1986
3 J V Hinshaw, Jr, *J Chromatogr Sci*, 25 (1987) 49–55
4 W Jennings and M F Mehzad, *J Chromatogr Sci*, 24 (1986) 34–40
5 K Grob, *Making and Manipulating Capillary Columns for Gas Chromatography*, Huthig, Heidelberg, 1986