Journal of Chromatography, 323 (1985) 81–87
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,415

Note

Performance of a moving belt liquid chromatography–mass spectrometry interface

J. VAN DER GREEF*, A. C. TAS and M. C. TEN NOEVER DE BRAUW
CIVO-Institutes TNO, P.O. Box 360, 3700 AJ Zeist (The Netherlands)
and
M. HÖHN, G. MEIJERHOFF and U. RAPP
Finnigan MAT, Barkhausenstrasse 2, 2800 Bremen 1 (F.R.G.)

In recent years, many different interfaces have been constructed[1] and successfully applied in liquid chromatography–mass spectrometry (LC–MS) studies. One of the most versatile types with respect to the choice of the ionization method is the moving belt. Both electron-impact (EI) and chemical-ionization (CI) methods can be used, but also "soft ionization" methods such as secondary-ion mass spectrometry (SIMS)[2,3] and fast atom bombardment (FAB)[4]. The construction of a moving-belt interface strongly influences its performance, as has been shown in a comparative study[5]. In this study, the performance of a moving-belt interface in combination with a magnetic mass spectrometer is investigated with respect to sensitivity, desorption characteristics and accuracy of determination in reversed- and normal-phase LC–MS experiments.

EXPERIMENTAL

A Finnigan MAT moving-belt interface was coupled with a double-focusing Finnigan MAT 8230 mass spectrometer equipped with a combined EI–CI source. The applications (experiments A–E) described were obtained with a Waters M6000 pump, a Waters U6K injector and a Nucleosil C_{18} column (300 × 4.0 mm I.D.) (A, B, C and E) and a Polygosil column (250 × 4.6 mm I.D.) (D).

The following mobile phases were used: methanol (A, B1 and C); methanol, containing 0.2% of ammonia (B2); acetonitrile–water–methanol (56:5:39) (E); and diisopropyl ether–hexane (10:90) stabilized with 10 mg/l of 2,6-di-*tert.*-butyl-4-methylphenol (D). The flow-rates were 0.6 ml/min (A, B2, C and E), 1.2 ml/min (B1) and 2.0 ml/min (D). Other conditions were: solvent evaporator, 180°C; source temperature, 150–220°C; ammonia CI, indicated source pressure $4 \cdot 10^{-1}$ Torr; intensity ratio $m/z = 35$ and $m/z = 52$ was 30:1 (A, B, C and E); EI = 70 eV (D).

RESULTS

(A) Deactivation of the belt

Deactivation of the belt with a 50-ppm Carbowax 20M solution reduces the memory effect as shown in Fig. 1 for single-ion monitoring (SIM) of the $[M + H]^+$

0021-9673/85/$03.30

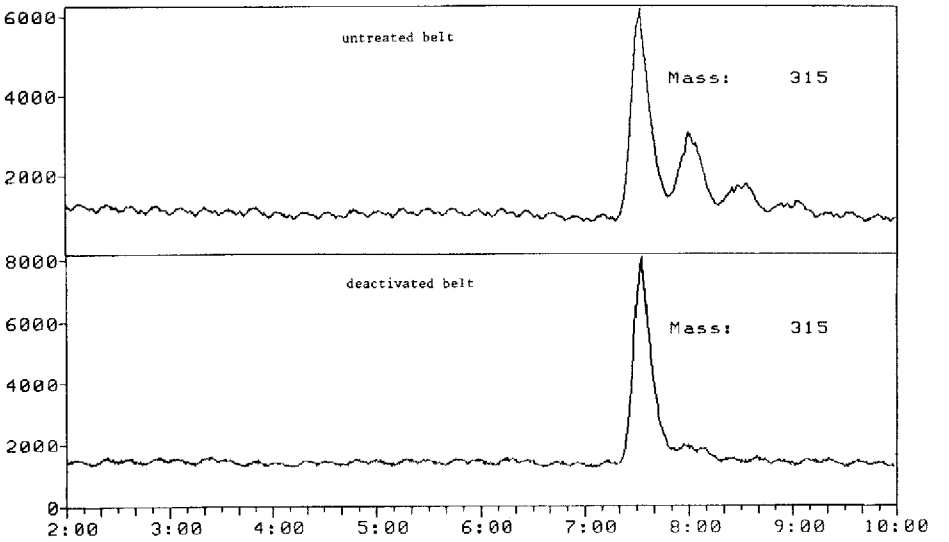

Fig. 1. SIM trace of the $[M + H]^+$ ion of progesterone under LC–CI-MS conditions before (A) and after (B) deactivation of the belt.

ion of progesterone, obtained prior to and after deactivation of the belt. Furthermore, deactivation improves the desorption characteristics of the belt. Two extreme cases are shown in Fig. 2a and 2b where CI spectra for bromazepam are reproduced, obtained by direct spotting on a deactivated belt and LC–MS analysis by using an untreated belt. As a function of the activity of the belt, spectra were obtained within the range of fragmentation (loss of hydrogen bromide) shown.

(B) Sensitivity

In the low-resolution mode, a detection limit of *ca.* 200 pg is obtained for progesterone and [3,4-$^{13}C_2$]progesterone under CI conditions while monitoring the

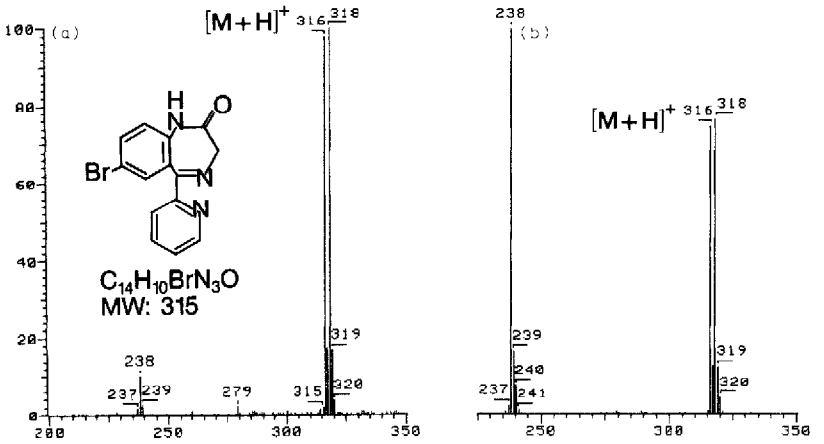

Fig. 2. CI spectra of bromazepam obtained by direct spotting on a deactivated: rated belt (A) and by LC–MS with an untreated belt.

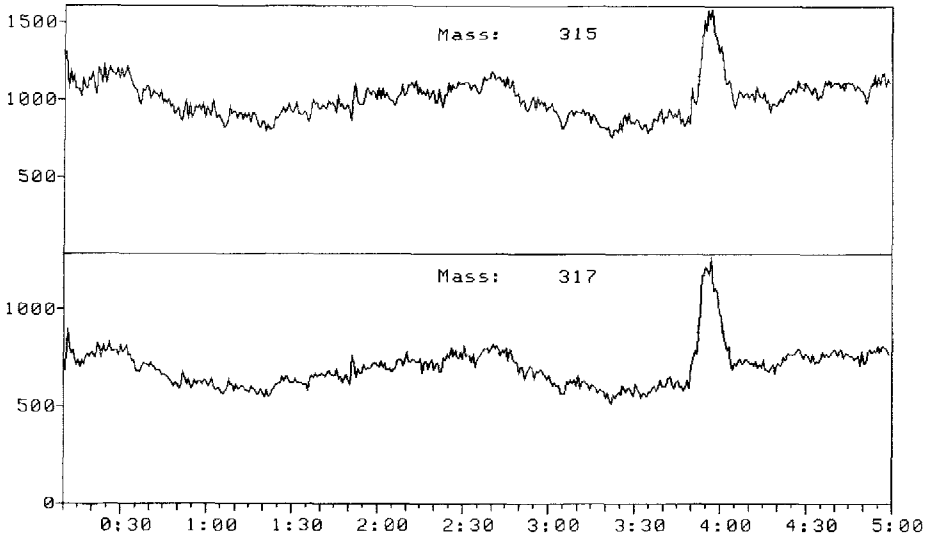

Fig. 3. LC–CI-MS analysis of 200 pg of progesterone and [3,4-$^{13}C_2$]progesterone each.

$[M + H]^+$ ions (Fig. 3). By using medium resolution (R = 4000), halogen-containing compounds can be separated from the background, as shown for bromazepam in Fig. 4. This results in a detection limit of 35 pg for this drug under LC–CI-MS conditions. With high-voltage scanning, accurate mass measurements (better than 5 ppm) were obtained for low-nanogram amounts of bromazepam in human serum extracts[6].

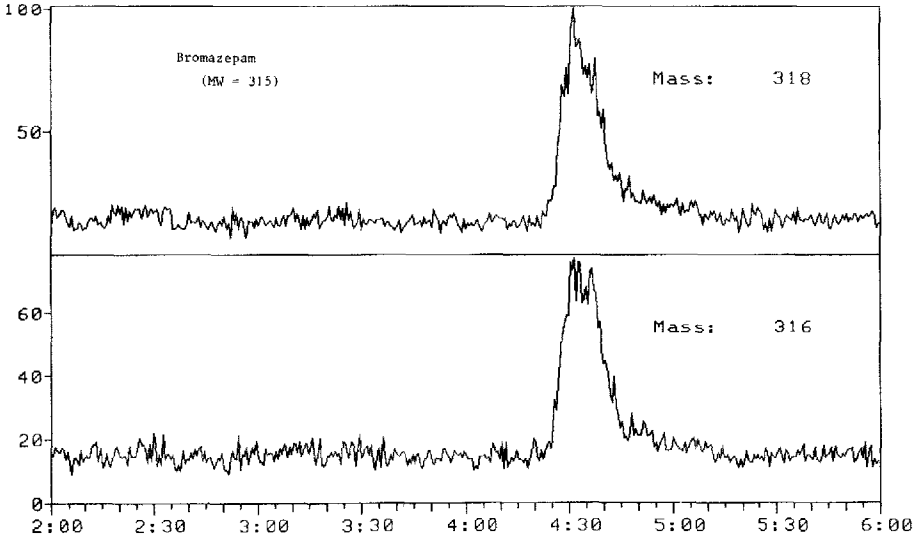

Fig. 4. LC–CI-MS analysis of 35 pg of bromazepam, using medium resolution.

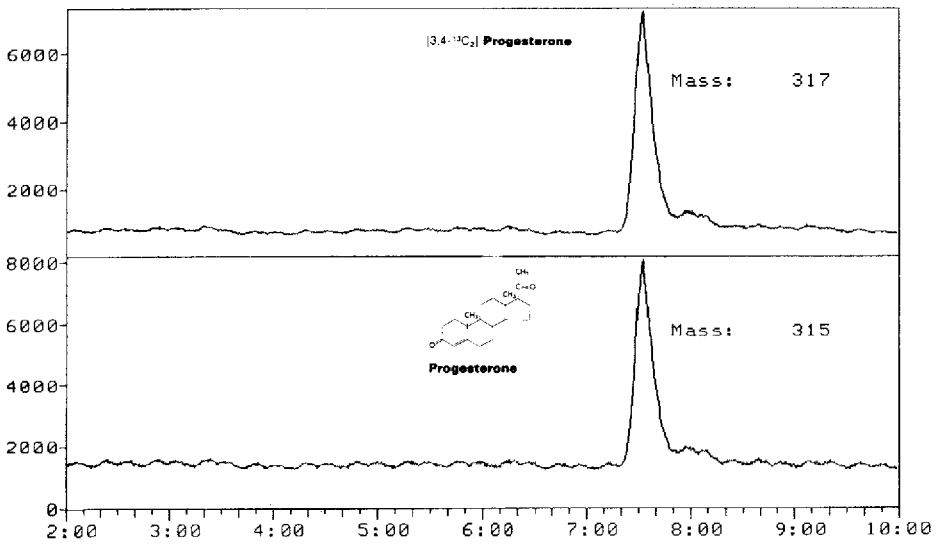

Fig. 5. LC–CI-MS isotope-dilution experiment for a standard solution containing 4.3 ng of progesterone and [3,4-$^{13}C_2$]progesterone each.

(C) Quantitation

Without derivatization, progesterone can be determined accurately by LC–CI-MS, by using isotope dilution as shown in Fig. 5 for a standard solution containing 4.3 ng of unlabelled and $^{13}C_2$-labelled progesterone. In Fig. 6, the determination of progestertone in a human serum extract is demonstrated. A concentration of 22.0 ± 0.5 ng/ml was found, which is in good agreement with the value of 18 ng/ml obtained by radio-immunoassay[7].

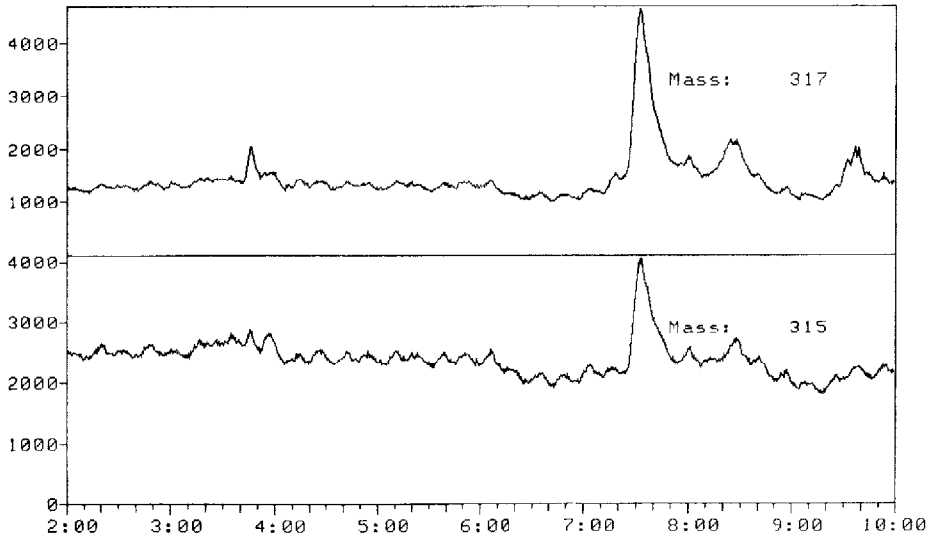

Fig. 6. Determination of progesterone in a human serum extract using isotope dilution.

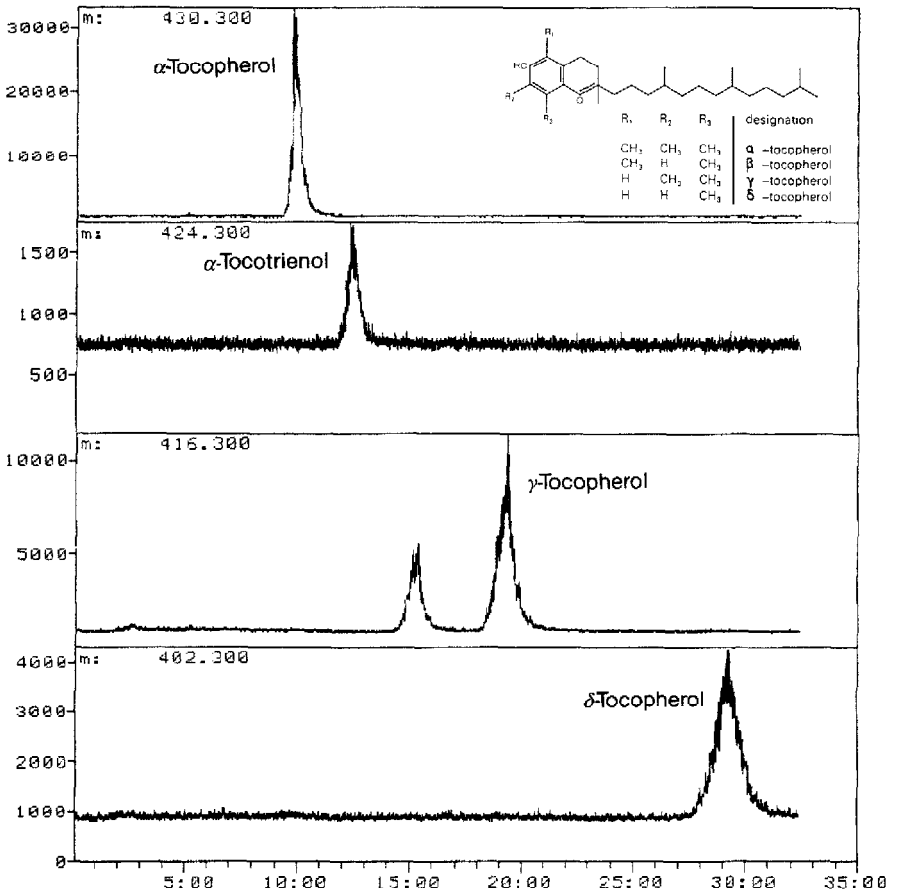

Fig. 7. LC–EI-MS multiple-ion detection of the molecular ions of tocopherols.

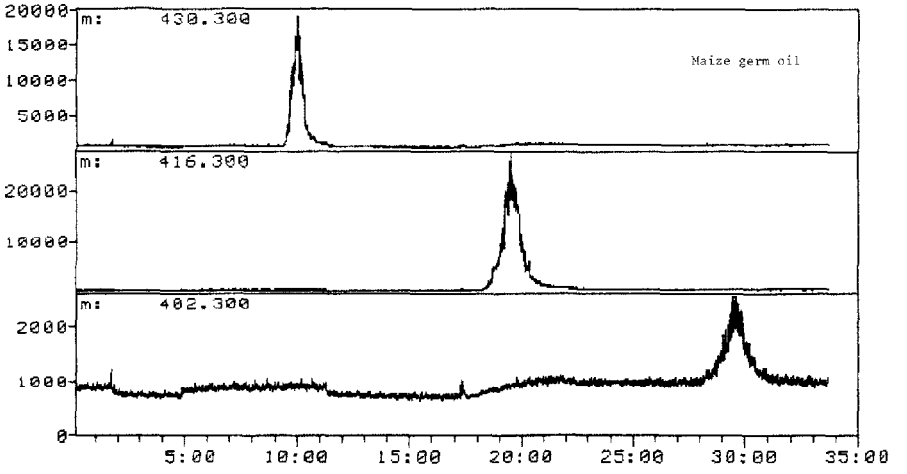

Fig. 8. LC–EI-MS multiple-ion detection of tocopherols in a maize germ oil.

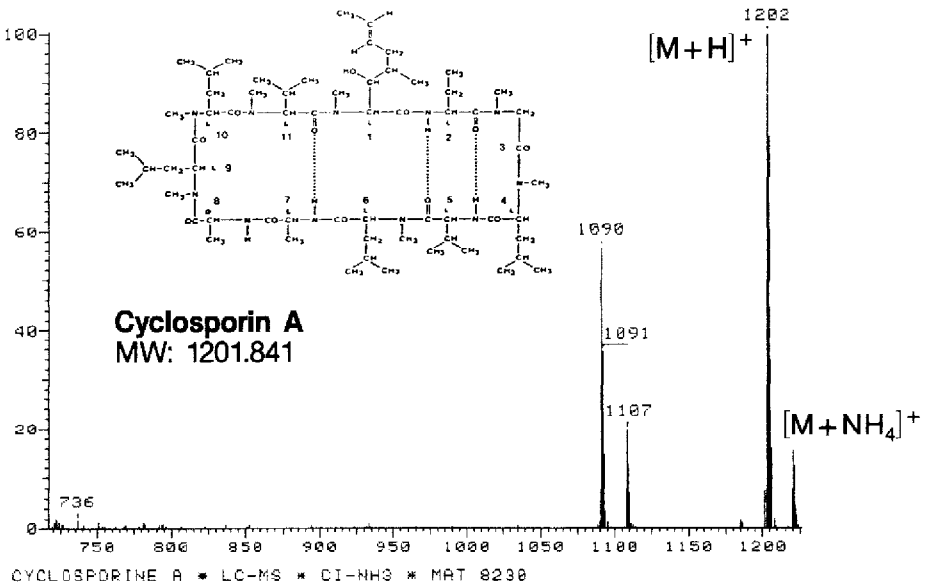

Fig. 9. CI spectrum of cyclosporin A obtained with LC–MS.

(D) Vitamin E analysis

Vitamin E analysis can be performed with a normal-phase system, as illustrated for a standard oil containing the various isomeric tocopherols (α, β, γ and δ) as well as added α-tocotrienol (Fig. 7). After 50-fold dilution of a maize germ oil, direct LC MS analysis results in the data of Fig. 8, enabling direct determination of some of the tocopherols. In order to avoid strong contamination effects due to the elution of glycerides, flushing of the LC effluent was effected between the eluting tocopherol isomers.

(E) Desorption characteristics

The cyclopeptide cyclosporin A gives abundant $[M + H]^+$ ions under LC–CI-MS conditions after deactivation of the belt (see Fig. 9). Without deactivation, the fragment at $m/z = 1090$ dominates the spectrum. This shows the ability of the moving-belt system to handle high-molecular-weight polar compounds.

CONCLUSIONS

The above described experiments carried out with a moving-belt LC–MS system show that:

(1) Deactivation positively influences the performance of a moving-belt LC–MS interface.

(2) Accurate and fast determination in the ppb range can be obtained with isotope dilution.

(3) Detection limits in the lower picogram range are possible.

(4) Reversed-phase as well as normal-phase systems can be applied.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. R. A. A. Maes, Mr. M. A. H. Rijk, Dr. J. Schrijver and Dr. A. J. Speek for providing samples and for their advice on the LC part of this work.

REFERENCES

1 D. E. Games, *Biomed. Mass Spectrom.*, 8 (1981) 454.
2 A. Benninghoven, A. Eicke, M. Junack, W. Sichtermann, J. Krizek and H. Peters, *Org. Mass Spectrom.*, 15 (1980) 459.
3 R. D. Smith, J. E. Burger and A. L. Johnson, *Anal. Chem.*, 53 (1981) 1120.
4 P. Dobberstein, E. Korte, G. Meyerhoff and R. Pesch, *Int. J. Mass Spectrom. Ion Phys.*, 46 (1983) 185.
5 D. E. Games, M. A. McDowall, K. Levsen, K. H. Schafer, P. Dobberstein and J. L. Gower, *Biomed. Mass Spectrom.*, 11 (1984) 87.
6 A. C. Tas, J. van der Greef, M. C. ten Noever de Brauw, R. A. A. Maes, T. Plomp, M. Höhn and U. Rapp, in preparation.
7 J. van der Greef, A. C. Tas, M. A. H. Rijk, M. C. ten Noever de Brauw, M. Höhn, G. Meyerhoff and U. Rapp, submitted for publication.