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Journal of Chromatography A, 847 (1999) 47–51

JOURNAL OF CHROMATOGRAPHY A

Short communication

# Gas chromatographic determination of galactose in milk Example of a switching valve used for the protection of the capillary column

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**Abstract**

Galactose, a marker of heat treatment, has been analysed in milk as pentafluorobenzyloxime acetate by gas chromatography with flame ionization detection using a simple switching valve system. The procedure did not entail any pre-derivatization clean-up for lactose elimination from the sample. In a short pre-column, reagent and lactose derivative excess were separated and the galactose and internal standard derivatives were transferred to the analytical column by a four-port valve. Thus, the analytical column was protected from overloading, so avoiding rapid deterioration. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Food analysis; Milk; Derivatization, GC; Galactose; Pentafluorobenzyloxime acetate

## 1. Introduction

Galactose (Gal) has been considered a marker of heat treatment of the milk [1] and its determination has been carried out by gas chromatography (GC) after treatment with silylating agents [2,3], which are known to give derivatives sensitive to moisture. In our laboratories a procedure based on reactions with pentafluorobenzylhydroxylamine (PFBHA) and acetic anhydride has been introduced to give stable derivatives suitable for the monosaccharide estimation [4,5]. Under the conditions suitable for monosaccharide separation, disaccharides are strongly retained and eluted only with high retention times as broad ghost peaks. Known procedures for the separation of milk lactose by ion-exchange resin [6] or precipitation in ethanol [7] are too time consuming to be suitable for a routine analytical method. In conclusion: in the GC analysis of milk Gal problems arise due to the high concentration of lactose which requires a large excess of the reagents and entails the overloading of the stationary phase and of the flame ionization detection (FID) system.

In order to isolate analytes from complex matrices multidimensional techniques have been introduced and new developments are recently reviewed [8]. In this brief communication, the use of a simple system consisting of a short pre-column and a switching

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PII: S0021-9673(99)00032-1

valve between the pre-column and the analytical one is described; by this means the analytical column was protected during the GC analysis of milk Gal.

## 2. Experimental

### *2.1. Apparatus and chromatographic conditions*

The used fused silica capillary columns (0.32 mm I.D.) contained Rtx-2330 stationary phase (0.2 μm thickness) (Superchrom., Milan, Italy). A Packard Model 470 A gas chromatograph equipped with split/splitless and OCI-3 on-column injectors and a FID system (Chrompack, Middelburg, Netherlands) was used. A Model 6C4WT Valco four-port switching valve (Superchrom) was placed in the oven. A short column (0.5 m) joined to a retention gap (3 m×0.32 mm) connected to the split/splitless injector, an analytical column (10 m) connected to the FID system, a short empty tube (4 m×0.32 mm) connected to the on-column injector and an open short empty tube were connected to the four ports. According to the two possible valve positions the eluate from the pre-column could exit directly in the oven or be transferred to the analytical column. The sample was injected in split mode (split ratio 1:20) into the pre-column when the valve was positioned so as to allow the eluate to be vented for 4 min at a flow-rate of 7 ml/min, while through the on-column injector a flow-rate of 1 ml/min was maintained in the analytical column. After 4 min the valve was positioned so as to allow the eluate from the pre-column to be transferred to the analytical column; after other 6 min the valve was positioned in the initial mode so as to allow the highly boiling

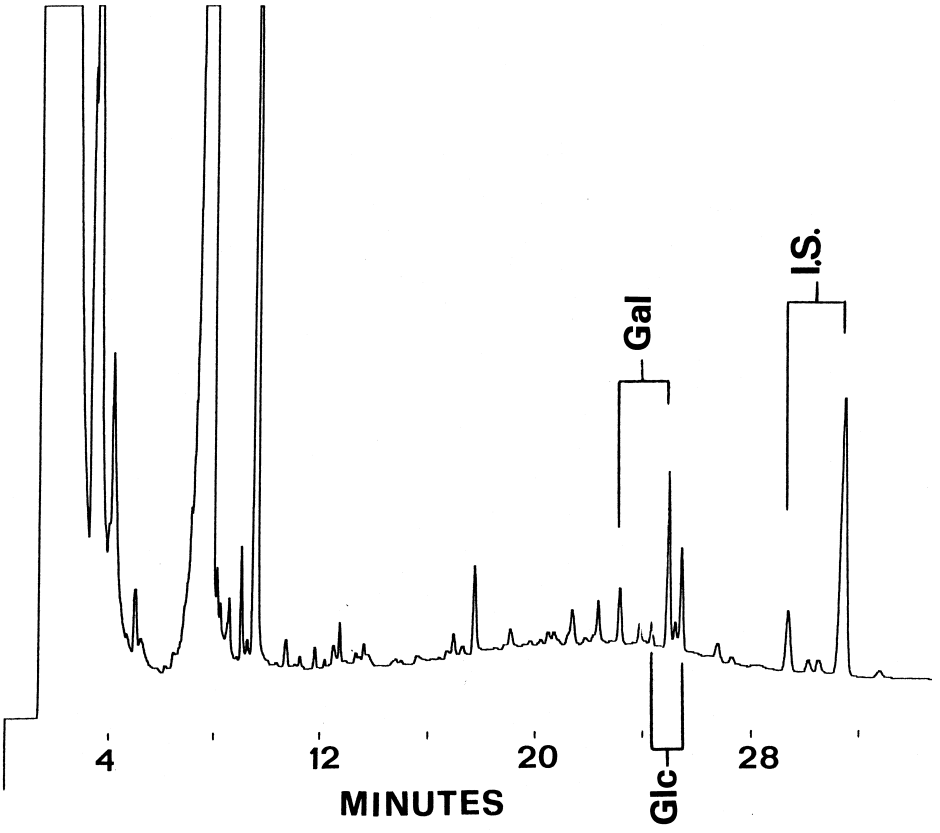


Fig. 1. GC profile of a milk sample subjected to the described procedure and directly analysed on a 10 m Rtx-2330 column joined to a 3 m retention gap. Carrier gas (helium) flow-rate was 1 ml/min.

compounds (as lactose) to be eliminated. The oven temperature was programmed from 120°C to 200°C at 10°C/min and from 200°C to 255°C at 3.5°C/min and finally maintained at 255°C for 12 min. The split/splitless injector temperature was maintained at 290°C.

### 2.2. Sample preparation

To commercial skim-milk (1 ml) 10% acetic acid was added (0.1 ml), the mixture was maintained at 40°C for 10 min, then 1 *M* sodium acetate (0.1 ml) was added and the mixture maintained at room temperature for 10 min. After centrifugation, the serum (0.1 ml) was withdrawn, 10 m*M* aqueous solution of D-glucoheptose, used as internal standard (I.S.) (0.03 ml) was added and the mixture brought to dryness under a nitrogen stream. The residue was reacted with a 25 mg/ml solution of PFBHA in pyridine (0.4 ml) for 20 min at 100°C and, after addition of acetic anhydride (0.5 ml), for another 20 min at 80°C. The sample was finally evaporated to dryness under a nitrogen stream; the residue was dissolved in ethyl acetate (0.2 ml) and injected into the gas chromatograph.

## 3. Results and discussion

In milk lactose molar content is about a hundred-times higher than Gal content and the use of a large PFBHA excess in order to assure the complete monosaccharide derivatization is noteworthy. In Fig. 1 a typical GC profile of a milk sample treated

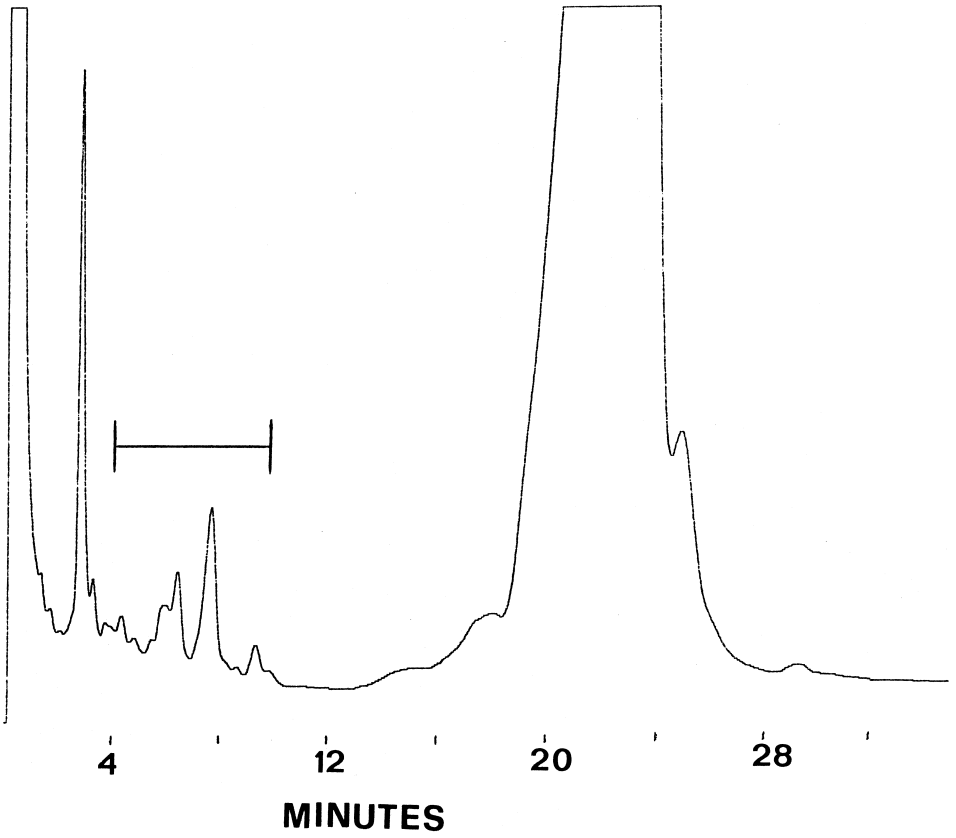


Fig. 2. GC profile of a milk sample analysed on a 0.5 m Rtx-2330 column joined to a 3 m retention gap. Helium flow-rate was 7 ml/min. The last large peak corresponds to lactose derivative. The bar corresponds to the time interval (4–10 min) between the two position changes of the switching valve in the subsequent multidimensional procedure.

according the described procedure and directly injected into a 10 m capillary column is shown. Each monosaccharide [Gal, glucose (Glc) and I.S.] gives two peaks corresponding to *syn*/*anti* isomers, as previously reported [4,5], while Gal silylated derivative is known to give three peaks [9]. D-Glucoheptose was preferred to 3-*O*-methylglucose, previously used as I.S., because of its better separation from the hexose zone. The reported profile was obtained with an almost new column: the large excess of PFBHA derivatives (peaks between solvent and 10 min retention time) and the broad ghost peak, probably due to lactose derivative injected in a previous run, are clearly shown. In our experience after 10–15 runs the efficiency of the column rapidly decreased and a new column was needed. In Fig. 2 a typical GC profile of a milk sample analysed with a short (0.5 m) column is shown. The large lactose headed peak clearly appears at the end of the profile, while monosaccharides were crowded in a short retention time interval (4–10 min). Under the finally chosen conditions the compounds eluted in this interval were allowed to be transferred into the 10 m analytical column by the switching valve. Fig. 3 shows a typical profile obtained after the installation of a four-port valve, where only the analytes of interest appear. In these new conditions only after 80–100 runs the efficiency decreased for the deterioration of

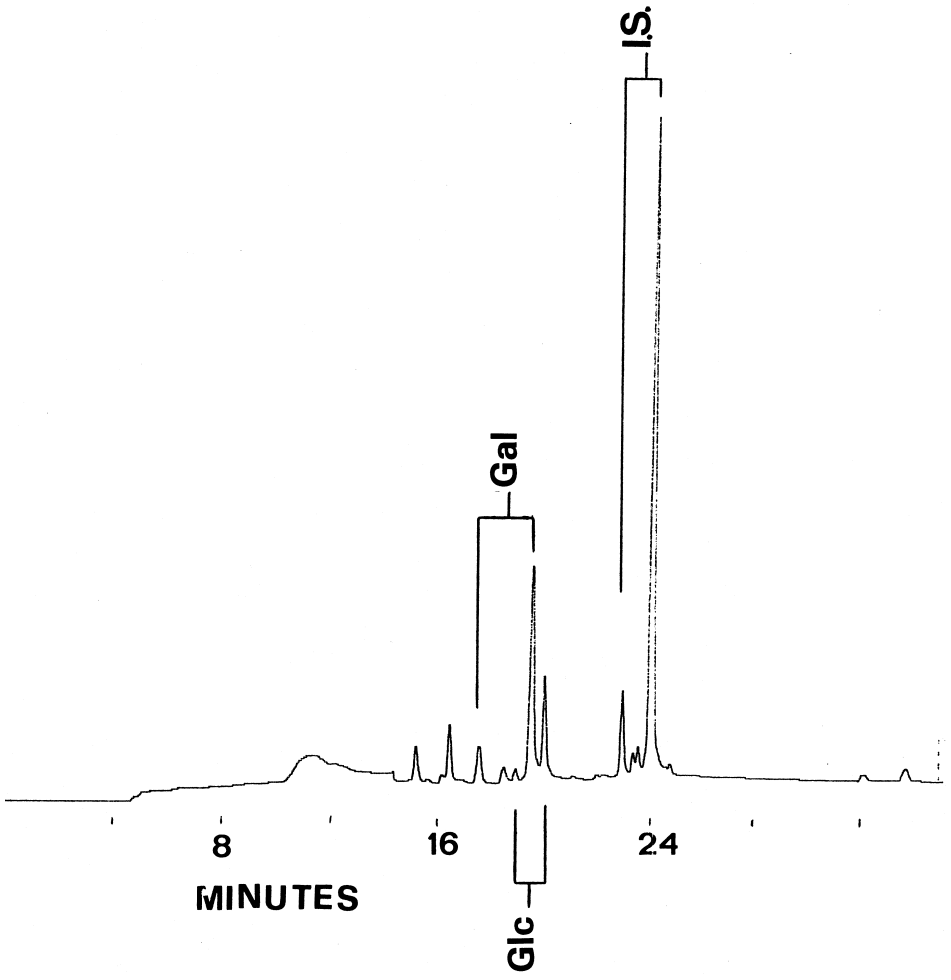


Fig. 3. GC profile of a milk sample analysed on a 10 m Rtx-2330 column by applying the described switching procedure, i.e. by allowing the mixture eluted from the pre-column in the time interval shown in Fig. 2 to be transferred into the analytical column.

the pre-column. Soon after the pre-column substitution the efficiency promptly returned acceptable. At present, after three substitutions of the pre-column, the analytical column used is still the same.

By subjecting different amounts of Gal (30–1000 nmol) and a same amount of internal standard (300 nmol) to the described procedure a linear relationship between the area ratios Gal/I.S. against Gal amounts was obtained according to the equation: area ratio=0.00323×Gal (nmol)−0.0367, ($r$= 0.996).

To test the precision of the method a commercial milk sample as analysed five times giving a Gal content of 1130±29 nmol/ml serum (RSD 2.7%).

In conclusion, the advantage of this procedure over other described ones can be summarized as follows: the installation of a simple four-port valve is demonstrated to be enough (a) to allow the use of pentafluorobenzyloxime acetate derivatives, which are more stable than the silylated ones, (b) to obtain satisfactory results without any previous purification of the samples so reducing the overall analysis time, (c) to protect the capillary column from overloading by reagents and interferents.

## Acknowledgements

The authors wish to thank Gino Micheletto (GSG Nuclear s.r.l., Bresso, Italy) for his helpful discussion and assistance. This work was supported by a grant from the Ministry of University and Scientific and Technological Research (M.U.R.S.T.) (funds to P.A.B.). L.M.C. was the recipient of a M.U.R.S.T. fellowship as a Ph.D. student in ''Control of total quality in zootechnic productions of food from animal sources''.

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