Journal of Chromatography, 579 (1992) 324–328
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6453

Short Communication

High-performance liquid chromatographic microassay for methyl ethyl ketone in urine as the 2,4-dinitrophenylhydrazone derivative

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(First received March 24th, 1992; revised manuscript received May 21st, 1992)

ABSTRACT

We report a high-performance liquid chromatographic procedure for determining methyl ethyl ketone in urine. The method is based on pre-column derivatization with 2,4-dinitrophenylhydrazine and liquid–liquid extraction of the derivative. The analyte is chromatographically separated from other urine constituents in less than 12 min and is detected by UV absorption at 360 nm. Peak height and concentration are linearly related. The relative standard deviation assessed for within-day imprecision was 3.2% at the 2.21 mg/l level. The mean analytical recovery from urines spiked with 1.0 mg/l ketone was 96.0 ± 6.1%. The simple sample handling, the small volume of urine required and the short amount of time taken for the whole procedure make it suitable for routine biomonitoring of exposure to methyl ethyl ketone in industrial workers. The concentration in urine from nine non-exposed controls was less than 0.1 mg/l. The concentrations measured in urine samples from 60 exposed workers ranged from 0.1 to 1.1 mg/l and from 0.3 to 3.6 mg/l at the before- and the end-shift collections, respectively.

INTRODUCTION

Methyl ethyl ketone (2-butanone, MEK) is a widely employed solvent in the chemical industry. It can be absorbed by inhalation and skin contact during exposure [1–3] and it is excreted unchanged in both expired air and urine [4]. Some metabolites of MEK have been found in the blood and urine of animals [5] after oral administration and in humans after MEK inhalation [6,7]. It has been reported that urinary MEK concentration is a reliable parameter for the evaluation for its environmental exposure [4].

Some gas chromatographic [1,6,8] or high-performance liquid chromatographic (HPLC) procedures [7,9] have so far been proposed for its determination.

We describe a rapid and simple procedure for

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0378-4347/92/$05.00

urine MEK determination using 2,4-dinitrophenylhydrazine (DNPH) as a derivatizing agent. The main advantages of this method are that no additional solid phase extraction step and only small samples are required.

EXPERIMENTAL

Chemicals and reagents

The following chemicals were used: cyclohexane, 2,4-dinitrophenylhydrazine, hydrochloric acid, methyl ethyl ketone and chloroform (Merck, Darmstadt, Germany; or Fluka, Buchs, Switzerland). Chromatography-grade acetonitrile (Merck) was used in the mobile phase.

A 250-mg aliquot of DNPH, recrystallized twice from HPLC-grade methanol, was diluted to 100 ml with 4.0 mol/l hydrochloric acid. The derivatizing solution was then purified by washing twice with 5.0-ml aliquots of chloroform; the solution, stored in the dark at 4°C and protected with a layer of cyclohexane, is stable for two weeks.

A 10.0 g/l solution of MEK in water was prepared and stored at −20°C. The concentrated standard was diluted daily with water to produce a 100 mg/l standard solution. Non-exposed urines supplemented with 0.2, 2.0 and 5.0 mg/l MEK were used as working standard solutions.

Urine samples

Urine samples from 60 rotogravure workers and from nine non-exposed control subjects were analysed. End-shift samples were collected at the work place on Fridays, before-shift samples on Mondays. Dark glass containers were used and urine samples were stored at 4°C and analysed within three days.

Derivative preparation

A 500-μl aliquot of urine sample or standard, 500 μl of derivatizing solution and 1.0 ml of cyclohexane were placed into a 2.5-ml polyethylene capped vial. The mixture was shaken and placed in the dark for 60 min at room temperature. The mixture was shaken again and, after centrifugation at 1000 *g* for 5 min, the organic layer was transferred into a glass test vial. The cyclohexane was evaporated to dryness in a water bath at 37°C under a weak oxygen-free helium stream and the residue redissolved in 500 μl of acetonitrile. A 20-μl aliquot of the reconstituted extract was then injected into the chromatograph.

Apparatus and chromatographic conditions

A Perkin-Elmer (Monza, Italy) quaternary pump 620 liquid chromatograph equipped with an octadecylsilyl LiChrosorb RP-18 (5 μm) column (250 × 4 mm I.D.) (Merck) connected to a Perisorb RP-18 (30–40 μm) (Merck) guard column (30 × 4 mm I.D.) and a Perkin-Elmer diode array UV–VIS 235 detector set at 360 nm were employed for chromatographic analysis. The detector was operated at 0.02 absorbance units full scale. A Perkin-Elmer ISS-100 autosampling injector was used. The chromatographic procedure and the data management were controlled by a PC AX2 computer (Epson Italia, Milan, Italy) provided with expert software (Analyst, Perkin-Elmer); chromatograms were printed with an Epson FX-850. Isocratic elution was performed at a flow-rate of 1.5 ml/min with acetonitrile–water (55:45, v/v) as the mobile phase.

Quantification

Each sample or standard was analysed in duplicate. The MEK-DNPH derivative was identified by its retention time. Peak height was used for the estimation of MEK concentration by referring it to the external standard calibration.

RESULTS

Aliquots of 500 μl of two urine samples from non-exposed subjects spiked with 0.2 and 5 mg/l MEK were mixed with 500 μl of DNPH (1.25 g/l), left to react in the dark at room temperature, and analysed as described above. The kinetics of derivatization was monitored and no increase in MEK-DNPH peaks was observed after 60 min of reaction time. Furthermore, we observed that a single extraction step with cyclohexane was able to quantitatively remove the derivative from the aqueous phase.

Fig. 1 shows chromatograms of a urine sample from a non-exposed subject, the same urine spiked with 2.0 mg/l MEK, and a urine sample from an exposed person.

The reaction between MEK and DNPH gives rise to two distinct geometric *syn* and *anti* isomers, which are resolved into two distinct peaks with retention times (t_R) of 8.8 and 9.7 min, re-

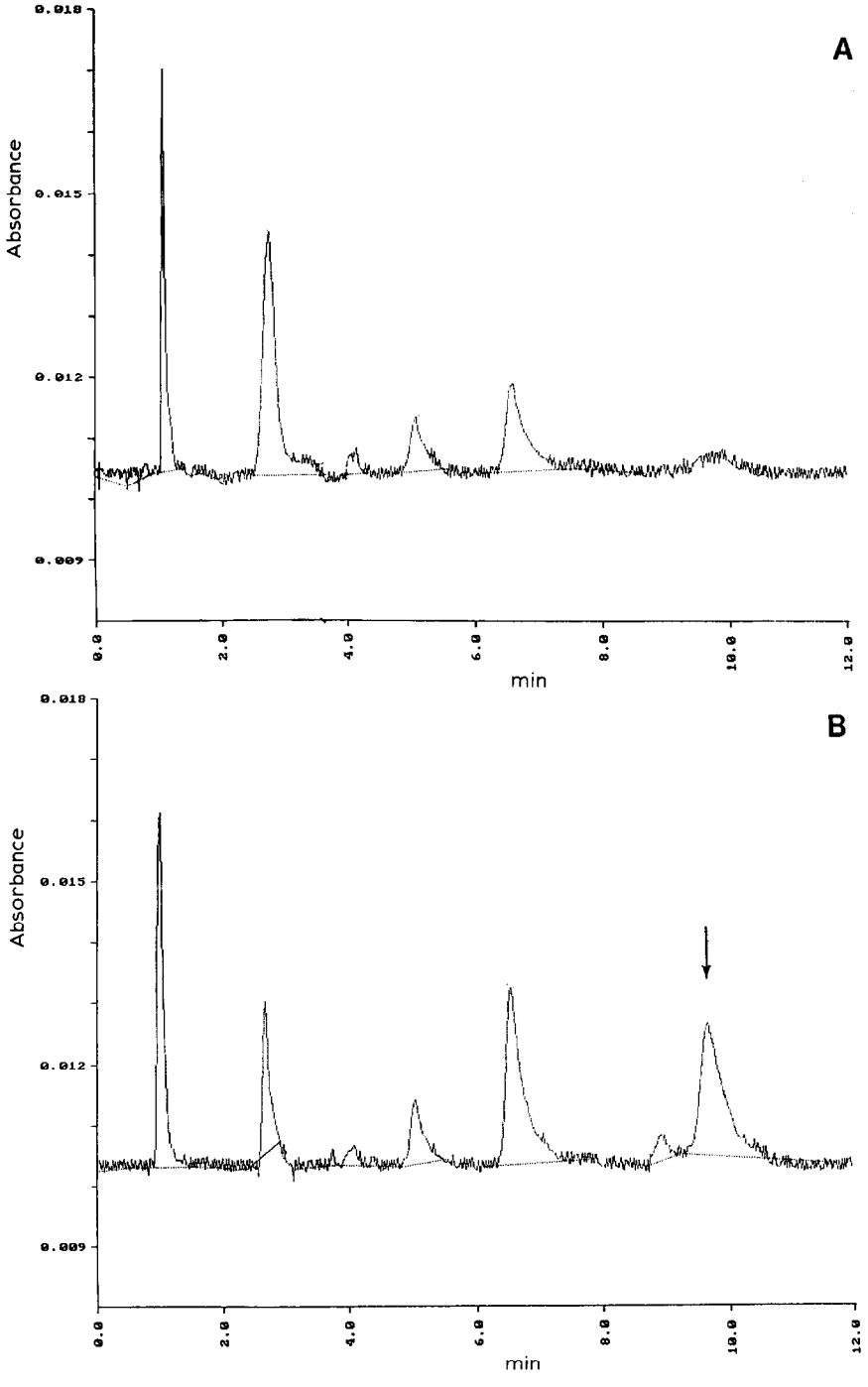

Fig. 1.

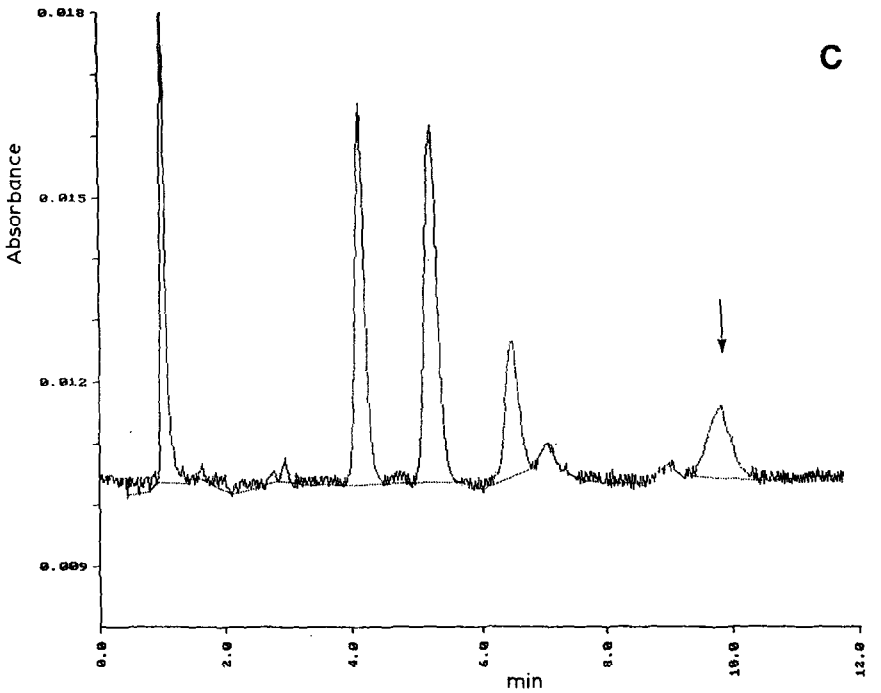

Fig. 1. Chromatograms of (A) urine from a non-exposed subject, (B) the same urine supplemented with 2.00 mg/l MEK and (C) end-shift urine sample from an exposed person, 1.05 mg/l.

spectively. The main peak (t_R = 9.7 min), which was used for calculations, is five-fold higher than the other and the height ratio, in the 0.2–5.0 mg/l range, is constant (5.11 ± 0.04, mean ± S.D., n = 20).

Reproducibility was tested with six repeated urine determinations to give coefficients of variation (C.V.) of 6.2 and 3.2% at the MEK levels of 0.21 and 2.21 mg/l, respectively. The repeat values from three exposed urine samples containing 1.7, 1.8 and 3.1 mg/l MEK, after storage for three days in the dark at 4°C, were unchanged (p = not significant, paired t-test).

The calibration curve was linear in the 0.1–5.0 mg/l range and the detection limit of the recommended procedure was 0.1 mg/l at a signal-to-noise ratio of 5:1.

The accuracy was tested by adding 1.0 and 2.0 mg/l MEK to five urine samples; mean recoveries of 96.0 ± 6.1% and 98.5 ± 4.6%, respectively (mean ± S.D.), were obtained.

The MEK concentration in nine non-exposed control urines was less than 0.1 mg/l. The concentrations measured in urine samples collected from 60 exposed workers ranged from 0.1 to 1.1 mg/l (0.52 ± 0.29, mean ± S.D.) and from 0.3 to

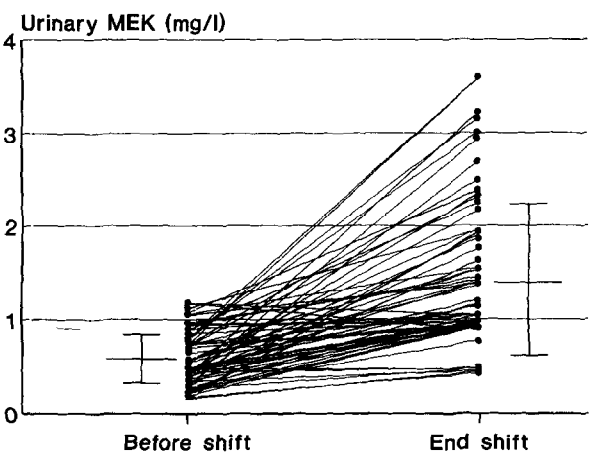

Fig. 2. MEK concentration measured in urine samples collected from 60 exposed workers before the shift on a Monday and after the shift on a Friday; means and standard deviations are shown.

3.6 mg/l (1.38 ± 0.84, mean ± S.D.) at the Monday before- and the Friday end-shift collection, respectively ($p < 0.001$, paired t-test) (Fig. 2).

CONCLUSIONS

A liquid–liquid extraction procedure, followed by liquid chromatographic separation, has been developed for the determination of MEK in urine. The isocratic chromatographic step takes 12 min to complete and uses a simple mobile phase composition. The extraction is simple to perform and is not time-consuming. No improvement in the recovery yields was obtained either by additional cyclohexane extraction steps or by increasing the organic-to-aqueous volume ratio. The injection of a greater amount of sample extract could be used to increase the sensitivity, but this shortens the column life without significantly improving the suitability of the screening procedure.

The main advantages of the described procedure compared with previously HPLC published methods are the use of small amounts of urine [9] and the fact that additional purification of the organic phase is unnecessary [7]. The HPLC procedure is sensitive, accurate, suitable for large-scale routine analyses and its application could be extended to the homologous series of compounds.

ACKNOWLEDGEMENT

The authors wish to thank Miss Antonella Schifone for her valuable work on the laboratory tests for this study.

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