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Diuretic Screening in Human Urine by Gas Chromatography Mass Spectrometry. Part 2 **

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Abstract

A simple and efficient procedure has been developed for the derivatisation of diuretic agents in human urine by direct extractive alkylation and their detection by GC-MS. The procedure is an improvement over previous extractive alkylation methods due to the development of a simple clean up step using a macroreticular acrylic copolymer (SM-7 resin) to remove the coextracted phase transfer reagent from the organic phase after derivatisation.

Introduction

Extractive alkylation is a convenient means of derivatising a wide range of acidic drugs before gas chromatographic (GC) analysis. By performing extractive alkylation directly on biological fluids the extraction and derivatisation of these drugs can be carried out quickly in a single step under mild reaction conditions. The disadvantage of using highly lipophilic phase transfer reagents in direct extractive alkylation reactions is the large quantity of the reagent which is coextracted into the organic phase.In a previous communication [1] we described a GC-MS procedure to screen for diuretic agents in human urine using direct extractive alkylation with THA+ as the phase transfer reagent and toluene or dichloromethane as the organic phase. After relatively few injections the pyrolysis product from the residual salts of tetrahexylammonium cation (THA+) caused a rapid deterioration in the efficiency of the capillary column with analyte retention times slowly increasing and the chromatograms displaying broad tailing fronts with continued column use. To avoid these problems an efficient clean up procedure is required in order to remove the coextracted salts of THA+ from the toluene after direct extractive alkylation. We describe

a new, efficient and simple clean up procedure based on solid phase extraction using a macroreticular acrylic copolymer (SM-7 resin) to remove the coextracted salts of THA+

Experimental

Screen for diuretics

To 1ml urine add
25μl of 6M NaOH
150μl of 0.2M tetrahexylammonium hydrogen sulphate
100μl 10ppm mefruside as internal standard
5mls of 0.5M methyl iodide made up in toluene
shake at room temperature for 20mins
centrifuge for 5mins at 1000rpm
remove toluene layer and pass through 2.5-3cm bed of SM-7 resin in pasteur pipette
evaporate toluene to dryness
redissolve the residue in 100μl of toluene

inject 2μl onto gc/ms.

GCMS conditions.
GC/MSD HP5880A/ HP5970
Column is HP Ultra 2 (SE54 Cross linked) 12 metre, 0.22mm diameter with 0.33μm film.
Carrier gas - helium at ca 0.8 ml/min split 1:8
Temperature program - 158° to 315° at 30°/minute then 315° for 5.5mins
injector temp 280°, transfer line 300°

Screen using two characteristic ions per compound.

Preparation of the SM-7 sorbent and the columns

The fines were removed by suspending the commercially available SM-7 sorbent in methanol and decanting the supernatant. This procedure was repeated until the supernatant was clear. The sorbent was then stored as a slurry under methanol until use.

To prepare the columns disposable glass pipettes (I.D. 6mm) were fitted with small plugs of silanised glass wool to act as bed supports and with a second pipette the resin slurry was added until columns of length 2.5-3.0cm were obtained (SM-7 resin 200-400 mesh, Bio-Rad Laboratories). Before use the columns were conditioned with 2ml of toluene.

Results and discussion

The Chromatogram was obtained after direct extractive alkylation on a composite urine sample consisting of urines taken from volunteers who ingested a single oral dose of acetazolamide (250mg), probenecid (500mg), dichlorphenamide (50mg), furosemide (50mg) and hydrochlorothiazide (50mg). The methyl derivatives of the diuretics have eluted with the toluene while the THA+ salts have been extracted by the SM-7 resin.
The pre-prepared SM-7 resin columns may be regenerated and reused by eluting the adsorbed salts of THA+ with 2ml of methanol and conditioning the columns with 2ml of toluene before the addition of the next sample. With this regeneration procedure we did not observe any carry over of the methyl derivatives from one sample to the next.

Detection limit

The detection limit was determined with the mass selective detector operated in the selected ion mode and was defined as the concentration of analyte that gave a signal to noise ratio of 3. The limits of detection for 12 diuretics are given in Table 1.

Recovery

To demonstrate the method recovery 8x1ml aliquots of urine were spiked to 2.5μg/ml with probenecid, dichlorphenamide, furosemide and hydrochlorothiazide and taken through the direct extractive alkylation procedure. The yields for the four diuretics were compared with pure solutions of tetramethyldichlorphenamide, trimethylfurosemide, tetramethylhydrochlorothiazide and the methyl ester of probenecid which were synthesised. The recovery and standard deviation for the four diuretics are shown in Table 2.

Conclusions

We have introduced a new more efficient procedure for the removal of the salts of THA+ from the toluene phase after performing extractive alkylation directly on urine samples. This simple clean up procedure overcomes the chromatographic problems caused by the coextraction of these salts resulting in improved signal to noise ratios and enabling

extractive alkylation to be more easily applied to the routine GC analysis of acidic drugs in biological fluids.

Chromatogram

TIC GC-MS profiles after performing direct extractive alkylation on two urine samples and extracting the THA+ salts from the toluene with a 2.5-3.0cm column of SM-7 resin. (1) blank urine sample; (2) composite urine sample from subjects who ingested a single oral dose of acetazolamide, probenecid, dichlorphenamide, furosemide and hydrochlorothiazide. Peaks A= methyl ester of indole-3-acetic acid; B= caffeine; C= unknown; D= monomethylated acetazolamide; E= trimethylated acetazolamide; F= methyl ester of probenecid; G= tetramethylated dichlorphenamide; H= trimethylated furosemide; I= dimethylated mefruside (internal standard); J= tetramethylated hydrochlorothiazide. Under the experimental conditions that were used any trihexylamine from the pyrolysis of THA+ salts would have eluted at 4.06 minutes.

Reference

1. A.M.Lisi, G.J.Trout and R.Kazlauskas, J. Chromatogr., 563 (1991) 257.

TABLE 1

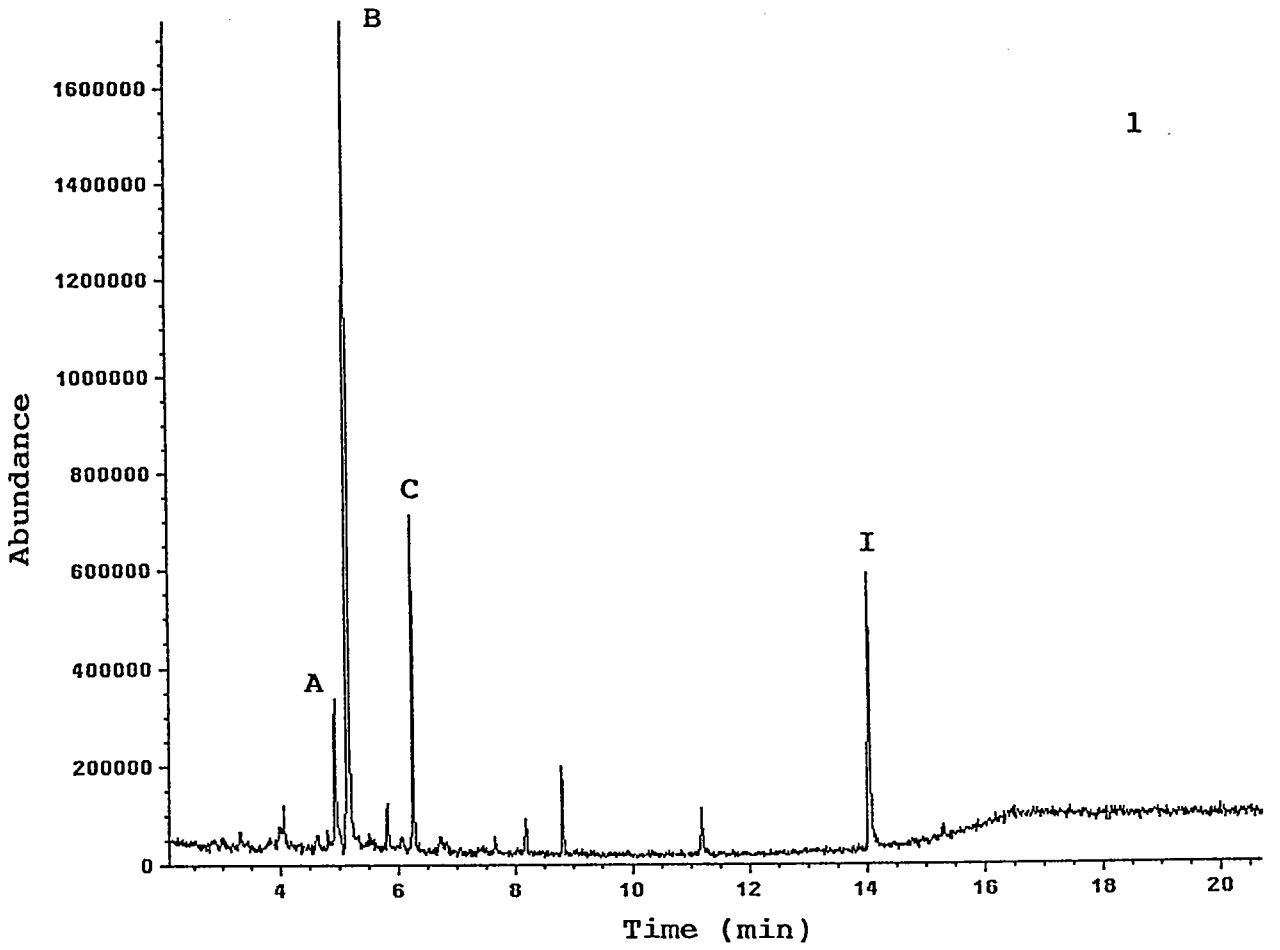
DETECTION LIMITS FOR 12 DIURETICS

Diuretic	Detection limit (ng/ml)
Acetazolamide	50
Probenecid	10
Dichlorphenamide	25
Hydroflumethiazide	10
Furosemide	10
Chlorthalidone	10
Bumetanide	10
Hydrochlorothiazide	50
Quinethazone	10
Bendroflumethiazide	10
Metolazone	10
Cyclopenthiazide	10

TABLE 2

RECOVERY OF FOUR DIURETICS FROM 1ml OF URINE SPIKED TO 2.5ug/ml

Diuretic	% Recovery	Standard deviation (n=8)
Probenecid	93	± 4
Dichlorphenamide	93	± 5
Furosemide	80	± 7
Hydrochlorothiazide	79	± 11



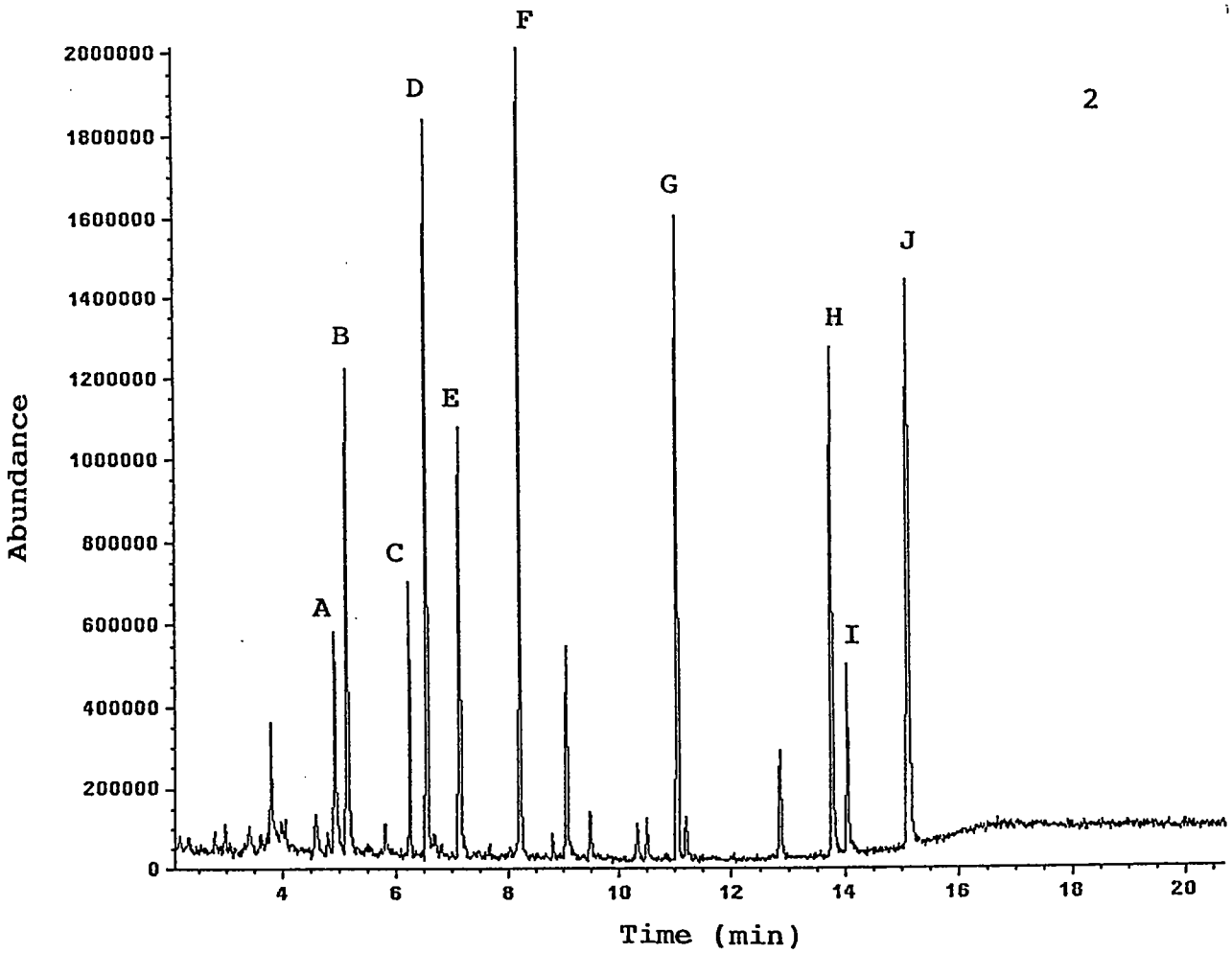


Figure 2