

## BONDED PHASE OF SILICA AND CARBOALKOXYALKYL SILANES FOR SOLID PHASE EXTRACTION

This is a divisional of co-pending application Ser. No. 731,530, filed on May 7, 1985, now U.S. Pat. No. 4,640,909.

### FIELD OF THE INVENTION

This invention relates to solid phase extraction packing material especially such packing material useful for the cleanup of urine samples for analysis of cannabinoids in urine.

### BACKGROUND TO THE INVENTION

Detection of marihuana use through analysis of biological samples, such as urine or blood, is coming into more widespread use. Because use of urine samples involves a non-invasive and more convenient process, detection of metabolites of  $\Delta$ -9-tetrahydrocannabinol in urine has begun to find greater popularity. Additionally, the use of such assay procedure has begun to develop greater importance and more widespread use not only because of its use to initially detect marihuana users but as an adjacent of drug counseling programs as a screening procedure to monitor compliance with withdrawal procedures and continued abstinence from marihuana use.

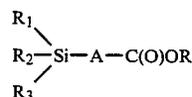
Of the several metabolites of  $\Delta$ -9-tetrahydrocannabinol found in urine the major is 11-nor- $\Delta$ -9-tetrahydrocannabinol-9-carboxylic acid, hereinafter referred to as THC-COOH, in either its free or conjugated (glucuronide) form. Various non-automated processes exist for detection of THC-COOH in urine, such as thin layer chromatography, gas chromatography, gas chromatography/mass spectrometry, radioimmunoassay, enzyme multiplied immunoassay and more recently high performance liquid chromatography (HPLC). However, such processes are quite labor intensive and due to the numerous and varied interferents in the urine sample are quite cumbersome to carry out. Additionally, it is difficult to measure THC-COOH in urine because of the complex nature of this matrix. The extraction of THC-COOH from urine is rendered more difficult because THC-COOH is one organic acid among a large number and variety of organic acids present in urine. A number of these organic acids have chromatographic properties which are similar to THC-COOH and will interfere with its measurement. Therefore, in order to be able to obtain a meaningful and relatively quick measurement of THC-COOH in urine one must be able to selectively extract it from the urine sample.

Current techniques for screening total urine samples for the presence of THC-COOH are generally either by thin layer chromatography or the Enzyme Multiplied Immunoassay Technique (EMIT) of Syva Company. Once a positive sample is detected a confirmational analysis is performed, usually by gas chromatography/mass spectrometry. However, with adequate cleanup of the urine sample, that is concentration of THC-COOH in the urine sample, and the use of an internal standard, confirmation of HPLC is possible. Thus, a great need exists for a much more satisfactory method of cleanup of urine samples to concentrate THC-COOH present in said samples. A bonded phase chromatographic packing that uniquely and specifically extracts THC-COOH from human urine would be highly desirable. More-

over, a bonded phase that is specific enough for THC-COOH yet permits selective elution of THC-COOH from the column without removing the impurities from the column or selective elution of the impurities from the column without removing the THC-COOH would be most desirable. A bonded phase that provides a purified urine extract clean enough to permit a more sensitive analysis of THC-COOH by confirmational methods, such as by HPLC, is greatly needed. Impure extracts also result in high and noisy baselines that decrease the capability for detecting low levels of THC-COOH. Cleanup of urine samples sufficient to detect low levels of THC-COOH would be most desirable.

### BRIEF DESCRIPTION OF THE INVENTION

In accordance with the invention, a bonded phase permitting the extraction of a more highly purified or concentrated form of THC-COOH from human urine is provided by the carboxyl free reaction product obtained from the reaction of silica gel or controlled pore glass, with carboalkoxyalkyl silanes of the formula



in which R is an alkyl radical of from 1 to 3 carbon atoms, A is an alkylene radical of from about 2 to 4 carbon atoms, and  $R_1$ ,  $R_2$  and  $R_3$  can be the same or different and are selected from halogen, alkoxy of from 1 to 6 carbon atoms, alkoxy alkoxy of from 2 to 5 carbon atoms and alkyl of from 1 to 3 carbon atoms, with the proviso that at least one of  $R_1$ ,  $R_2$  and  $R_3$  is a group other than an alkyl group. With such bonded phase silica products one obtains a much purer extract of THC-COOH from urine than from previously used or available bonded phases and this permits quantitation at much lower levels of THC-COOH and also more accurate measurement of THC-COOH. Additionally using this bonded phase a much simpler and more rapid extraction of THC-COOH is obtained.

### DETAILED DESCRIPTION OF THE INVENTION

The carboalkoxyalkyl silanes reacted with silica gel or controlled pore glass can be any silane of the hereinbefore set forth formula. In said formula halogen is preferably chlorine, R is preferably methyl and A is preferably ethylene. Most preferably  $R_1$ ,  $R_2$  and  $R_3$  are methyl or chlorine, most preferably each chlorine. As examples of such silanes to be reacted with silica gel or controlled pore glass there may be mentioned, for example, carbomethoxyethyl trichlorosilane, carbomethylethyl methylchlorosilane, carbomethoxyethyl dimethylchlorosilane, carboethoxypropyl, trichlorosilane, carbomethoxybutyl trichlorosilane, carbopropoxyethyl trichlorosilane, carbomethoxyethyl trimethoxysilane, carbomethoxyethyl trimethoxymethoxysilane, carbomethoxyethyl triethoxysilane and the like.

The reaction product is useful as a column packing in liquid chromatography for the purification and separation of THC-COOH from human urine especially in high performance liquid chromatography (HPLC) applications.

Such bonded phase silica products are obtained by reaction of silanes of the hereinbefore set forth formula