

BULLETIN

OF THE

KOREAN CHEMICAL SOCIETY

VOLUME 16, NUMBER 2
FEBRUARY 20, 1995

BKCS 16(2) 77-196
ISSN 0253-2964

Communications

Determination of Cimetidine in Human Urine by Column Switching HPLC with UV-detection

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Received September 16, 1994

Cimetidine, 1-cyano-2-methyl-3[2-(5-methylimidazol-4-yl)-methylthio ethyl]-guanidine, is widely used in the treatment of gastric and duodenal ulcer as a histamine H₂-receptor antagonist. Spectrophotometric^{1,2} and polarographic³ methods have been reported for the determination of cimetidine but these methods may not be specific. High performance liquid chromatographic analyses⁴⁻⁶ for the measurement of cimetidine in biological samples have also been proposed. However, conventional HPLC techniques are not in themselves suitable for trace analysis of cimetidine in biological fluids and should include either additional sample pretreatment steps. For the determination of cimetidine by HPLC, earlier studies⁴⁻⁶ of off-line sample pretreatment procedures have been described. Methods implying only deproteinization of biological fluids without enrichment would not have been sensitive enough for the purposes of trace analysis. The sample treatment in these methods is tedious and may be accomplished by evaporation of solvent or by multiple liquid-liquid extraction steps and protein precipitation. In practice, however, these procedures are time consuming, incomplete extraction and substance losses are further drawbacks of these tedious operations. Therefore these procedures exhibited low precision and sensitivity. Column switching HPLC by on-line purification without an extraction steps using precolumn enrichment proved to allow the problems above to be solved⁷⁻¹¹.

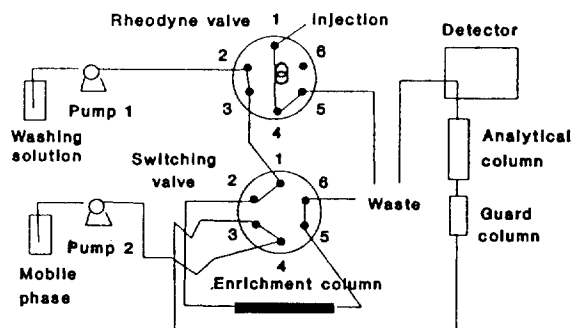
We have validated an column switching HPLC method. The objective of this communication is to describe an automated HPLC method for the determination of cimetidine with direct injection of human urine using a column switching technique for on-line sample clean-up.

Cimetidine (100.0%) was kindly supplied by Yuhan (Seoul, Korea). HPLC grade acetonitrile and methanol were pur-

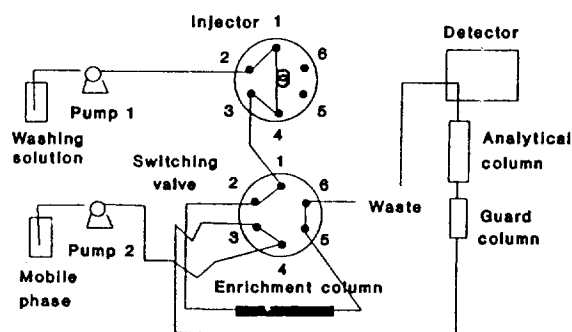
chased from Merck. Water used in the experiments was distilled-deionized and then purified using a E-pure water purification system (Barnstead). All other chemicals were of analytical grade from Aldrich.

UV-visible spectra were recorded on a Hewlett Packard HP8452 diode array spectrophotometer linked with HP-IB interface. Two solvent delivery systems (SP-8800 ternary pump from Spectra Physics, USA and LC-9A isocratic pump from Shimadzu, Japan) were used in conjunction with Rheodyne 7125 injector and Spectra 100 UV detector (Spectra Physics, USA). Data handling was performed by means of SP4600 Datajet integrator (Spectra Physics, USA). Spheri-5 RP C₁₈ (Spectra Physics, 30 mm×4.6 mm i.d., 5 micron) column was used for on-line clean-up. Throughout this experiment, HPLC analysis was performed on an octyl C₈ (Supelcosil LC-8-DB, 75 mm×4.6 mm i.d., 5 micron, Supelco) column as analytical column and octyl C₈ (Supelcosil LC-8, 20 mm×4.6 mm i.d., 5 micron, Supelco) column as guard column.

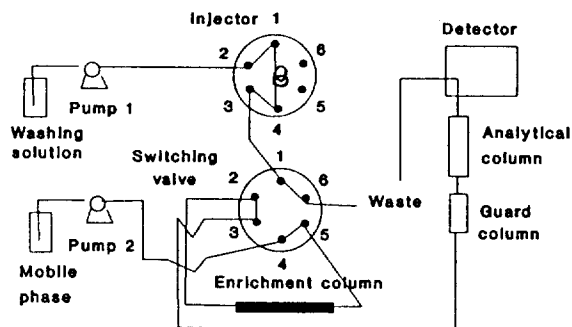
A schematic diagram of the column switching HPLC system is given in Figure 1. An enrichment column was mounted independent of injector between port 2 and port 5 of a high pressure six port switching valve (Whitey, USA). A guard column was mounted between port 3 of the switching valve and the analytical column in order to protect the analytical column from interfering endogenous constituents in urine. At the first loading step, washing solution of 0.02 M ammonium acetate buffer (pH 6.71) was delivered by Pump 1 at a flow rate of 1.0 mL/min. Aliquots (10 µL) of urine sample spiked cimetidine (20 µg/mL) were injected by a Rheodyne 10 µL loop injector onto the precolumn. At the second washing step, the enrichment column had to be washed for ten min. to eliminate interfering compounds before elution took place. Lipophilic compounds, including cimetidine were retained on the enrichment column, while proteins and other polar components of urine were washed out to waste. A guard column and the analytical column were previously equilibrated with mobile phase of 0.02 M potassium phosphate solution (pH 4.60-4.75) containing 10 v/v % acetonitrile delivered by Pump 2 at a flow rate of 1.0 mL/min. At the final elution step, the elaborated switching device operated in a back flush mode. At 10 minutes after injection, switching valve was actuated manually to port 6 position



A) Loading step



B) Washing step



C) Elution step

Figure 1. Schematic experimental set-up for column switching HPLC and successive operations performed during steps of operation.

from port 1 and then the retained cimetidine was backflushed from the enrichment column to the guard column and analytical column with mobile phase at the ambient temperature. The eluted cimetidine from the enrichment column were separated in the analytical column. Meanwhile enrichment column was reconditioned with washing solution for the next injection. Column effluent was monitored at 220 nm, since this corresponds to the wavelength of maximum UV absorbance of cimetidine.

The whole procedure was conducted on-line in the HPLC system and did not increase handling and analysis time. These procedure was efficient for increasing the sensitivity of UV detection when standards were tested. Representative chromatograms from the urine sample are shown in Figure 2.

The recovery of a fixed amount of drug in urine during

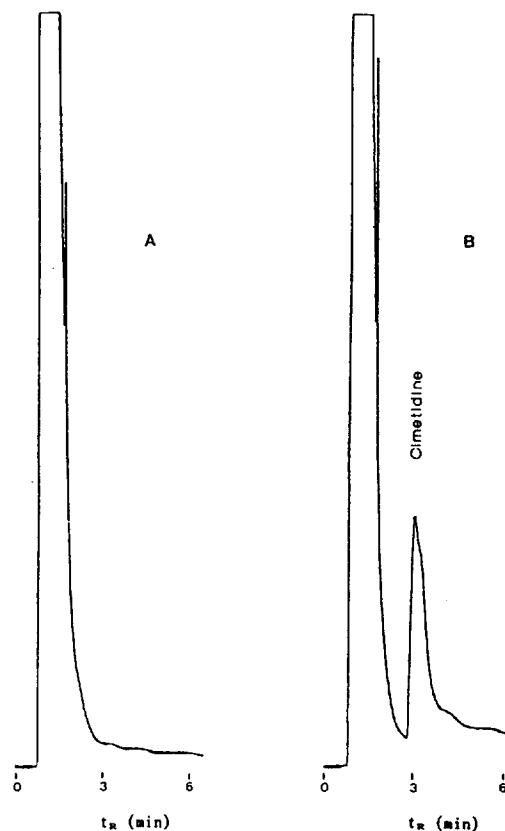


Figure 2. Chromatograms of A) a human drug free urine and B) the same urine spiked cimetidine (20 $\mu\text{g}/\text{mL}$) by column switching technique.

the course of the column switching was tested by comparing solid phase extraction. The recovery of cimetidine by solid phase extraction HPLC using ODS silica cartridge (Sep-Pak C_{18}) from drug free urine was 78.92 (± 0.22)% and reproducibility (defined as the coefficient of variation) of five replicate analyses was 0.09%. This results obtained are in good agreement the report by Imamura *et al.*⁵ but poor than present column switching HPLC. The recovery of column switching HPLC was 87.63 (± 0.22)% and reproducibility was 0.04%. This result indicate that on-line purification without an extraction process allows to prevent substance losses. The method was linear over the concentration range of 1-30 $\mu\text{g}/\text{mL}$ ($r=0.997$) based on the peak area. Detection limit (S/N ratio=3) of column switching HPLC was 1.0 $\mu\text{g}/\text{mL}$ while solid phase extraction HPLC was 2.5 $\mu\text{g}/\text{mL}$. Compared to other methods of sample pretreatment, the on-line column switching method is superior concerning time of work and analysis. Both sample treatment and clean-up procedure are performed automatically within the analytical run-time. The on-line column switching HPLC is a precise, reliable, easy to implement, and work saving method for clean-up and analysis of cimetidine in biological samples.

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Formation of Octa-Chlorinated Dibenzo-p-dioxin and Its Thermal Decomposition Products from Pyrolysis Reaction of Pentachlorophenate

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Received September 29, 1994

Usually octa-chlorinated dibenzo-p-dioxin (octa-CDD) exists as impurities in technical penta-chlorophenol and in chlorinated phenoxyacetic acids.¹ In addition, octa-CDD has been found in emission from municipal waste incinerators²⁻⁵ and in several environmental samples including sediment or sea water with relatively higher concentration over other chlorinated dibenzo-p-dioxins.^{6,7} The main polychlorinated dibenzo-p-dioxins (PCDDs) including octa-CDD found in the fly ash are similar to those formed in the pyrolysis of commercial chlorophenols.⁸ Therefore, the burning of chlorophenols is of interest in connection with incineration conditions designed to destroy waste PCDDs.

In this study, octa-CDD and some of PCDDs were produced by microscale pyrolysis of pentachlorophenate (PCP) at relatively high temperature. The mixture of octa-CDD and its thermal decomposition products was separated and identified by high resolution gas chromatography-mass spectrometry (GC-MS). Specifically, potassium pentachlorophenate in the reaction tube containing alumina beads was pyrolyzed at 700 °C, at which most of the incinerators operate. After

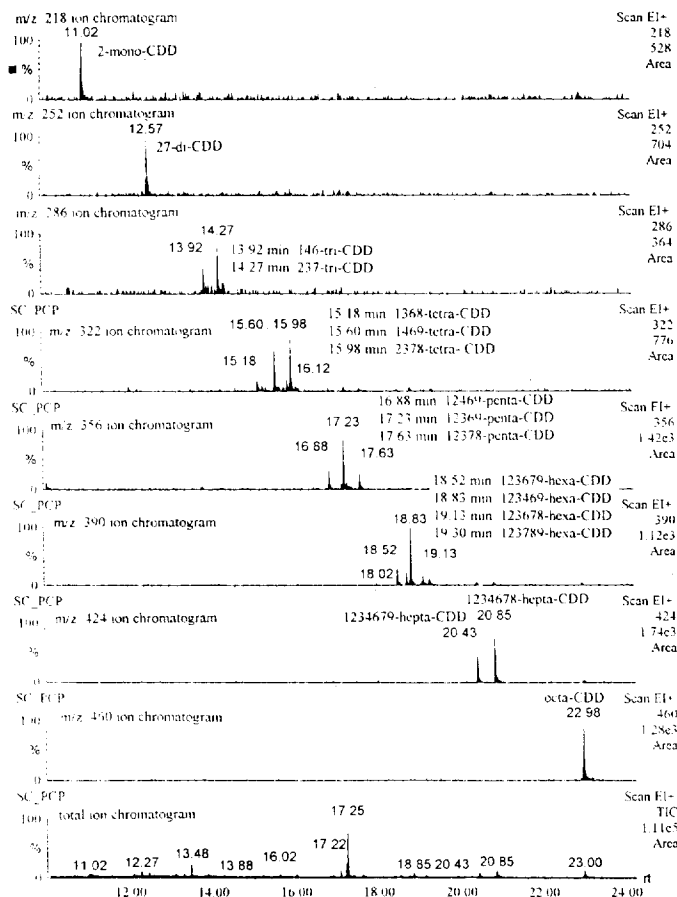


Figure 1. Typical total ion chromatogram and ion chromatograms of pyrolysates obtained from the pyrolysis of pentachlorophenate. Chromatographic conditions as follows: injection temperature 280 °C; splitless mode; column SPB-5 30 m×0.25 mm; oven temperature: initial temperature 100 °C held for 2 min and increased at 20 °C/min to 200 °C and then programmed at 10 °C/min to 300 °C.

pyrolysis, the pyrolysates adsorbed on the surface of alumina beads and glass reaction tube were dissolved in methylene chloride by sonication for 10 min. The dissolved products were placed on an alumina column, eluted with 20 mL of methylene chloride, and then evaporated under a nitrogen stream. The unreacted reagents such as PCP and other polar compounds were readily removed from this column by clean-up procedure. The extracts were analyzed by high resolution capillary column GC-MS (Fisons Inst., Trio-1000, Manchester, UK).

As seen from the total ion chromatogram and ion chromatograms shown in Figure 1, octa-CDD and several PCDDs were detected, but no polychlorinated dibenzofurans (PCDFs) were found.⁹ The formation of octa-CDD can be explained as two-step condensation process through an aromatic substitution reaction *via* nona-chlorodiphenyl ether phenoxide as an intermediate, as indicated in Scheme 1. However, the formation mechanism of the dechlorinated products from octa-CDD is not yet equivocal. It could be explained by two pathways. One pathway is that octa-CDD formed from the condensation of pentachlorophenate can be converted into hepta-, hexa- and lower CDDs by sequential thermal dechloro-