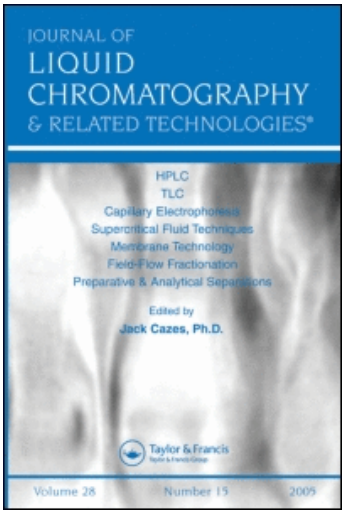

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Estimation of High-Performance Liquid Chromatographic Retention Indices of Glucuronide Metabolites

John K. Baker[a]
[a] Department of Medicinal Chemistry School of Pharmacy, University of Mississippi, University, MS

To cite this Article Baker, John K.(1981) 'Estimation of High-Performance Liquid Chromatographic Retention Indices of Glucuronide Metabolites', Journal of Liquid Chromatography & Related Technologies, 4: 2, 271 — 278
To link to this Article: DOI: 10.1080/01483918108064815
URL: http://dx.doi.org/10.1080/01483918108064815

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(2), 271-278 (1981)

ESTIMATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC RETENTION INDICES OF GLUCURONIDE METABOLITES

John K. Baker
Department of Medicinal Chemistry
School of Pharmacy
University of Mississippi
University, MS 38677

ABSTRACT

The retention indices of several glucuronide metabolites and their parent compounds were measured using a reversed-phase HPLC system. It was found that the typical glucuronide metabolite had a retention index 244 ± 31 units lower than the parent compound.

INTRODUCTION

Though glucuronide formation is the most common pathway to the production of mammalian urinary metabolites of drugs and organic compounds, surprisingly little is known about the chromatographic properties of this important group of compounds. The most common approach to the identification and quantitation of these metabolites is to assay for the free aglycon following enzymatic or chemical hydrolysis of the sample and then subtract the quantity of free aglycon that was originally in the sample. Because of the high polarity of these metabolites, it is extremely difficult to extract these compounds from urine samples. Despite these difficulties, the intact glucuronides have been analyzed by

gas chromatography following derivatization of the carboxylic acid group with diazomethane and derivatization of the hydroxyl groups with N,O-bis(tri-methylsilyl) acetamide (1).

Direct analysis of the intact glucuronide without derivatization using high-performance liquid chromatography is a much more promising approach to the problem. The major objective of this study was to determine if the glucuronides could be chromatographed satisfactorily using reversed-phase columns and if the retention index of the metabolite can be predicted.

EXPERIMENTAL

Materials

The 2-keto alkane standards were obtained from Analabs (North Haven, Connecticut). Morphine and codeine were obtained from the Theta Corporation (Media, Pennsylvania). Morphine-3-glucuronide and codeine glucuronide were obtained from Applied Science Inc. (State College, Pennsylvania). Testosterone, testosterone glucuronide, 6-Bromonaphthol, 6-Bromonaphthol glucuronide, phenolphthalein, phenolphthalein glucuronide, 8-hydroxyquinoline, 8-hydroxyquinoline glucuronide, p-nitrophenol, and p-nitrophenol glucuronide were obtained from Sigma Chemical Company (Saint Louis, Missouri). All other chemicals and solvents were of reagent grade and were used without further purification.

Chromatography

A 3.9 mm i.d. x 30 cm C_{18} reversed-phase column (μ-Bondapak

C_{18}, Waters Associates, Inc., Milford) with a 10μm particle size was used for the study. The mobile phase flow rate was 2.0 ml/min and was comprised of 6.6 g K_2HPO_4, 8.4 g KH_2PO_4, 1.6 l CH_3OH, and 2.4 l H_2O. The pH of the mobile phase was 7.0 before the addition of the methanol.

A Waters Associates, Inc. M-6000 pump, U6K injector, and Model 440 dual wavelength ultraviolet detector were used. The first detector was operated at 254 nm, while the second detector (in series) was operated at 280 nm.

Retention Index Measurements

The basic construction and properties of the retention index scale have been previously reported (2). The capacity factor (k'_x) of the test compounds and standards were determined from the observed retention time (t_x) and the retention time of the solvent front (t_o). The index (I) of a given 2-keto alkane standard was, by definition, equal to 100 times the number of carbons in the standard. Thus, acetone was assigned a value of 300, and 2-butanone, 400. The index of a drug was calculated from the capacity factor observed for the drug (k'_x), the capacity factor for a 2-keto alkane standard eluting just before the test compound (k'_N), and the capacity factor of the next higher homologue (k'_{N+1}) using Equation 2.

$$k'_x = \frac{t_x - t_o}{t_o} \qquad \text{Eq. 1}$$

$$I = 100 \frac{\log k'_x - \log k'_N}{\log k'_{N+1} - \log k'_N} + 100N \qquad \text{Eq. 2}$$

RESULTS AND DISCUSSION

The retention times of the compounds and their glucuronide metabolite varied from about one minute for p-nitrophenol glucuronide to over one hour for testosterone. The difference in the retention time of the glucuronide metabolite and the aglycon varied considerably from compound to compound and this parameter would not be useful for the prediction of the retention time of the glucuronide derivative of some new test compound. However, when the HPLC retention index of the glucuronide and its aglycon were examined, a very clear pattern was observed (Table I). The retention index of the glucuronide was found to have a much lower retention index as one might expect for the highly polar glucuronide group and it was observed that the shift in retention index was very nearly the same for all of the glucuronides. The average value for the shift in retention index was found to be 244 ± 31 units. If this trend were to be extended to other drugs and organic compounds, one would predict that retention index of the glucuronide metabolite would be 244 units lower than that experimentally observed for the parent compound. The ability to predict the retention properties of the metabolite would be extremely useful because reference standards for the metabolites are difficult to obtain and they are rarely available in the early stages of the metabolism studies of a new compound.

TABLE I

Effect of Glucuronide Formation on the HPLC Retention Index of Drugs

Drug	Index	Shift
morphine	625	
morphine-3-glucuronide	361	-264
codeine	712	
codeine glucuronide	489	-223
testosterone	920	
testosterone glucuronide	710	-210
6-bromonaphtol	871	
6-bromonaphtol glucuronide	617	-254
phenolphthalein	740	
phenolphthalein glucuronide	508	-232
8-hydroxyquinoline	612	
8-hydroxyquinoline glucuronide	311	-301
p-nitrophenol	493	
p-nitrophenol glucuronide	270	-223
	Average =	-244 ± 31

In previous studies, it was found that the retention index of a test compound (I_x) could be predicted from the retention index experimentally observed for a related reference compound (I_{ref}) and the sum of the Hansch substituent constants (π_x) than are readily obtained from reference tables. The estimation of the retention index of the new compound was made using Equation 3 which was previously developed (3,4).

$$I_x = 200\ \pi_x + I_{ref} \qquad \text{Eq. 3}$$

Though this equation was not developed specifically for metabolism studies, the retention index of a metabolite could be estimated if the retention index of the parent compound were known and if the Hansch π values for all of the substituent changes were

available. Unfortunately, the π value for the glucuronide group was not available. However, if Equation 3 is used in the reverse manner, a 244 unit retention index shift would indicate that the glucuronide group would have a π value of - 1.22 ± 0.16 units. If more than one structural change occurred during the metabolic transformation of the drug, one would use the sum of the π values for each of the changes to estimate the retention of the final metabolite. All of the aglycons in Table I contain a hydroxyl group which was than conjugated to form the glucuride. For simple aromatic compounds that do not contain a hydroxyl group, the π value would be equal to the sum of the glucuronide group and the aromatic hydroxyl group (-0.67(5)) which would be -1.89. Thus, the estimated retention index of the glucuronide metabolites of non-hydroxylated aromatic compounds would be 378 units lower than the parent compound rather than the 244 units shown in Table I.

One of the major difficulties in the detection of drug metabolities in urine samples is that there are a large number of natural components in the urine that have strong UV chromophores. Unless one uses extremely selective extraction procedures, one is almost assured of finding a natural component with nearly the same retention time as the drug metabolite. If dual UV detectors are used in series, the absorbance ratio at the two wavelengths for the compound can be obtained with good precision and this measurement has been shown to be an extremely useful aid in the identification of the compound (6, 7).

TABLE II

The Relative Response of 254 nm and 280 nm Ultraviolet Detectors to Glucuronide Metabolites

Compound	A_{254}/A_{280}
morphine	0.77
morphine-3-glucuronide	0.50
codeine	1.27
codeine glucuronide	1.17
testosterone	ca.35
testosterone glucuronide	33.8
6-bromonaphtol	0.96
6-bromonaphtol glucuronide	1.08
phenolphthalein	1.23
phenolphthalein glucuronide	1.26
8-hydroxyquinoline	1.49
8-hydroxyquinoline glucuronide	0.56
p-nitrophenol	1.11
p-nitrophenol glucuronide	0.38

The absorbance ratio of the compounds and their glucuronide metabolites (Table II) was also found to be very useful in the characterization of the compounds in the present study. For those aglycons that have the hydroxyl group removed from the UV chromophore (codeine and testosterone) one would expect the glucuronide metabolite to have the same absorbance ratio which was observed. Most of the phenolic metabolite either showed a reduction in the absorbance ratio (morphine, 8-hydroxyquinoline, and p-nitrophenol) or remained unchanged (6-bromonaphthol and phenolphthalein) when conjugated with glucuronic acid.

Preliminary studies with codeine-6-glucuronide using the 40% methanol mobile phase given in the experimental have indicated that the glucuronide is poorly resolved from the natural constituents in

human urine. If the methanol content of the mobile phase was reduced to 20%, the glucuronide could be detected at 30 μg/ml in directly injected urine samples. If lower levels of the glucuronide are to be measured it will be necessary to remove interfering peaks that elute before and after the glucuronide.

ACKNOWLEDGEMENTS

This work was supported in part by a faculty research grant from the University of Mississippi and in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

REFERENCES

1. Billets, S., Lietman, P. S., and Fenselan, C., Mass Spectral Analysis of Glucuronides, J. Med. Chem., 16, 30 (1973).

2. Baker, J. K. and Ma, C. Y., Retention Index Scale for Liquid-liquid Chromatography, 169, 107 (1979).

3. Baker, J. K., Estimation of High Pressure Liquid Chromatographic Retention Indices, Anal. Chem., 51, 1693 (1979).

4. Baker, J. K., Skelton, R. E., Riley, T. N. and Bagley, J. R., Estimation of High Pressure Liquid Chromatographic Retention Indices of Narcotic Analgetic and related compounds, J. Chromatogr. Sci., 18, 153 (1980).

5. Tute, M. S., Principles and Practice of Hansch Analysis, Adv. Drug Res., 6, 1 (1971).

6. Krstulovic, A. M., Rosie, D. M. and Brown, P. R., Selective Monitoring of Polynuclear Aromatic Hydrocarbons by High Pressure Liquid Chromatography with Variable Wavelength Detector, Anal. Chem., 48, 1383 (1976).

7. Baker, J. K. Skelton, R. E. and Ma, C. Y., Identification of Drugs by High-Pressure Liquid Chromatography with Dual Wavelength Ultraviolet Detection, J. Chromatogr., 168, 417 (1979).