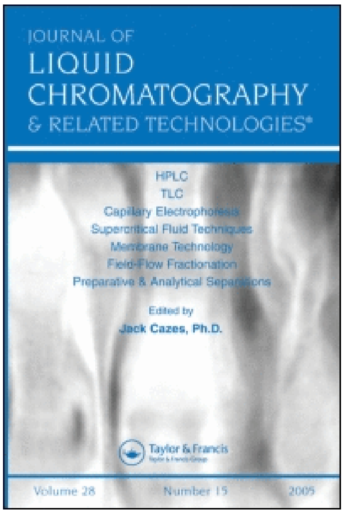

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STUDY OF HPLC RETENTION BEHAVIOUR OF FUROCOUMARINS IN PEUCEDANUM PALUSTRE WITH REFERENCE TO SOLVENT SELECTIVITY AND MOLECULAR CONNECTIVITY

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Abstract

The separation factor (α) and molecular connectivity indices (χ) up to the sixth order were calculated and compared with measured reversed-phase high pressure liquid chromatographic (HPLC) retention data for eight closely related furocoumarins from *Peucedanum palustre*. Retention measurements were performed over the capacity factor (k′) range 1-35 using organic solvent / water eluents containing acetonitrile, dioxan, ethanol, methanol, n-propanol and tetrahydrofuran. The solvents showed differences in the elution order of the furocoumarins. Decreasing the volume ratio (Φ) increased in many cases the separation factor. The compounds were divided into two groups; the low-order valence level indices and path/cluster type of the fifth order valence level indices best described the retention. As the high correlations between the calculated and observed retention indicate, retention could be predicted in six organic solvents with a high degree of accuracy by the molecular connectivity indices.

Introduction

Peucedanum palustre (L.) Moench has recently been shown to possess significant biological activity in inhibiting the KCl induced contractions of vascular smooth muscle (1), and in inhibiting prolactin release from rat pituitary tumor cells (2). Furocoumarins responsible for these activities have been isolated from the roots of *P. palustre* (3). The separation of the main coumarins in *P. palustre* can be carried out by various planar chromatographic methods using a normal phase (4). An isocratic HPLC method with a reversed phase has also been developed for the separation of the closely related eight furocoumarins in *P. palustre* using the "PRISMA"-model (5).

Chromatography is a dynamic equilibration process. At the molecular level, HPLC involves the distribution of solute molecules between a mobile phase and a stationary phase. In order to maximize the relative differences between the affinities of the mobile and stationary phases for sample components, one has to take into consideration the parameters that influence the separation factor, a. The separation factor is one of the most significant factors describing how well two compounds are separated from each other: $\alpha = k'_2/k'_1$, where k'_1 and k'_2 are the capacity factors of the last and the first eluted compounds, respectively (6).The mobile phase is, in turn, the chromatographic system parameter that can be most easily varied in order to optimize a for HPLC analyses.

The retention of compounds in HPLC analyses can be predicted using molecular connectivity indices, χ (7-9). The concept of molecular connectivity was introduced by Randic (10) and further developed by Kier and Hall (11). The χ terms are numerical values which are fundamental in defining and describing quantitatively the adjacency relationships in the molecular structure. When the nature of the atom is not taken into consideration the index is referred to as the connectivity level, χ. If it is, the index is described as the valence level, χ^v. Connectivity indices have been extended to include indices of different orders, as well as subgraphs composed of paths, clusters and path/clusters, which are described by the subscripts p, c, and pc, respectively.

The behaviour of heteroatom-containing molecules are sometimes difficult to predict on the basis of molecular connectivity indices. As shown by Lehtonen (12) the retention of oxygen-containing amines could not be predicted when analysed together with non-oxygen-containing amines. Kier and Hall (11) have improved the correlation between the water solubility of oxygen-containing compounds and the corresponding molecular connectivity indices by adding the vertex value, δ, of the oxygen atom to the regression equation for alcohols and ethers. A similar procedure gave high correlation for boiling points and partition coefficients in the case of primary, secondary and tertiary amines.

The aim of the present study was to examine the effect of six different organic solvents in water on the separation factor between eight furocoumarins, containing several oxygen atoms, found in *P. palustre* . The retention behaviour of the coumarins with reference to separation factor and molecular connectivity was studied under 36 different reversed-phase liquid chromatographic conditions.

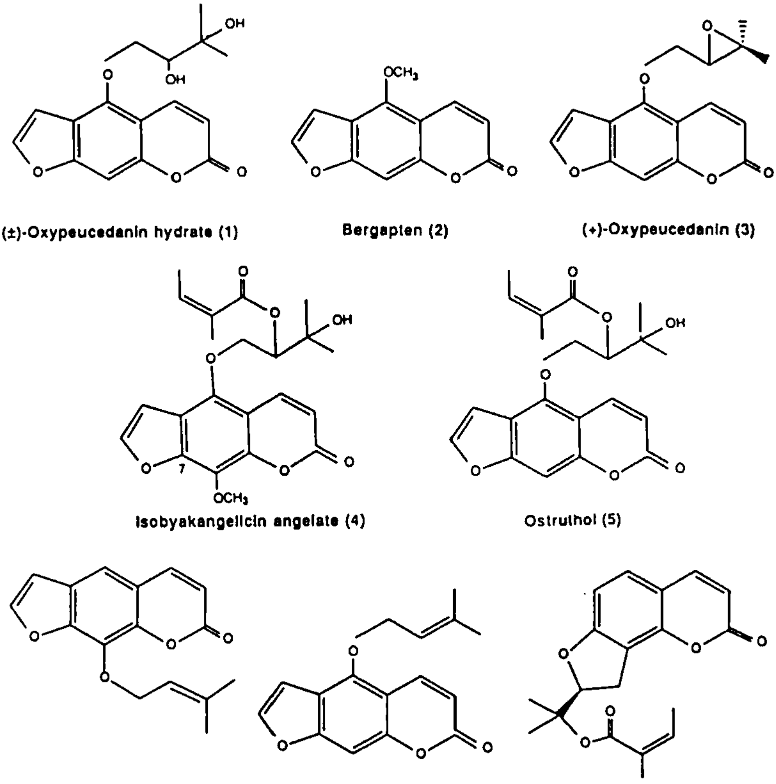
Experimental

Apparatus

A Waters 6000A liguid chromatograph equipped with a PU4020 UV detector (Pye Unicam LTD, UK) and Hewlett Packard 3390A integrator were used.

Chemicals

The furocoumarin isomers (Fig.1) (oxypeucedanin hydrate, oxypeucedanin, isobyakangelicin angelate, ostruthol, iso-imperatorin and columbianadin) were isolated and identified at the Department of Pharmacy, Federal Institute of Technology (ETH), Zurich, Switzerland (3). Bergapten and imperatorin were obtained from Roth (Karlsruhe, FRG). The water was distilled and deionised. All other solvents used were of HPLC quality (Rathburn, Walkerburn, UK).



(±)-Oxypeucedanin hydrate (1)

Bergapten (2)

(+)-Oxypeucedanin (3)

Isobyakangelicin angelate (4)

Ostruthol (5)

Imperatorin (6)

Isoimperatorin (7)

Columbianadin (8)

Figure 1. Structures of furocoumarins investigated

Chromatographic conditions

Column: A 100x4 mm i.d. cartridge (Knauer, Berlin, FRG) packed with Spherisorb ODS II, 3μm (Phase Separations, Queensferry, UK).

Mobile phase: acetonitrile, dioxan, ethanol, methanol, n-propanol, tetrahydrofuran each diluted with water (see Results and Discussion).

Flow rate: 1 ml/min; Detection: 320 nm; Sample amount: The samples were diluted to give the smallest detectable peaks at 320 nm.

Calculation of the molecular connectivity indices

The molecular connectivity indices for the furocoumarins were calculated using a self-made program running on BASIC, as has earlier been described for dansylamides (13). The following general equation proposed by Kier and Hall (11) was used for computation of an index of type t and order m .

$${}^{m}\chi_{t} = \sum_{j=1}^{{}^{m}n_{s}} {}^{m}c_{j} = \sum_{j=1}^{{}^{m}n_{s}} \left[\prod_{i=1}^{m} (\delta_{i})_{j}^{-1/2} \right]$$

where ${}^{m}c_{j}$ is the subgraph term for m:th order subgraphs and ${}^{m}n_{S}$, is the number of m:th order subgraphs.

Correlation of retention with the molecular connectivity indices

The IBM Statistical analysis System (SAS) RSQUARE procedure was used for determining the index best describing retention. The SYSREG procedure of the same system was used to determine the regression coefficients in linear regressions (14). The furocoumarins were divided into two groups according to their retention behaviour with reference to connectivity indices: group I (compounds 1, 3, 6 and 7; see Fig. 1) and group II (compounds 2, 3, 4, 5 and 8; see Fig. 1).

Results and discussion

Retention measurements were carried out for the closely related furocoumarins in volume ratios (*Φ*) of acetonitrile (46-36%) , dioxan (51-41%), ethanol (50-40%), methanol (64-54%), n-propanol (35-25%) and tetrahydrofuran (39-29%), each in six steps, by decreasing the amount of organic solvent in water by 2% of volume.

Figure 2 and 3 illustrate the influence of Φ on the separation factor (α) between eight furocoumarins for dioxan and tetrahydrofuran. In dioxan-water mixtures (Fig. 2) the separation factor for the first two eluting compounds, oxypeucedanin hydrate (1) and bergapten (2), remained constant at 3.0. The α-value between (+)-oxypeucedanin (3) and imperatorin (6) strongly increased from 1.6 to 2.3 when Φ was decreased from 0.51 to 0.41. Separation factor for compounds 5 and 6, as well as for compounds 7 and 4, slightly decreased along with

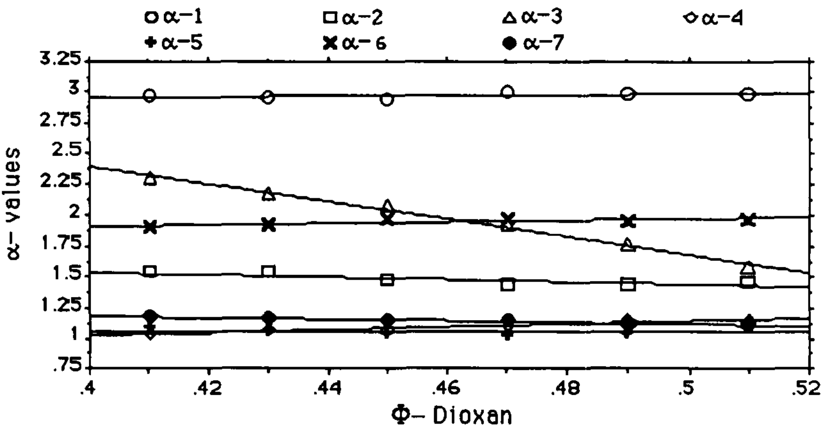


Figure 2. The effect of Φ-Dioxan on column separation factor (α) (α-1=k′ of compound 2/k′ of compound 1, α-2=k′3/k′2, α-3=k′6/k′3, α-4=k′5/k′6, α-5=k′4/k′5, α-6=k′7/k′4, α-7=k′8/k′7; compounds see Fig. 1).

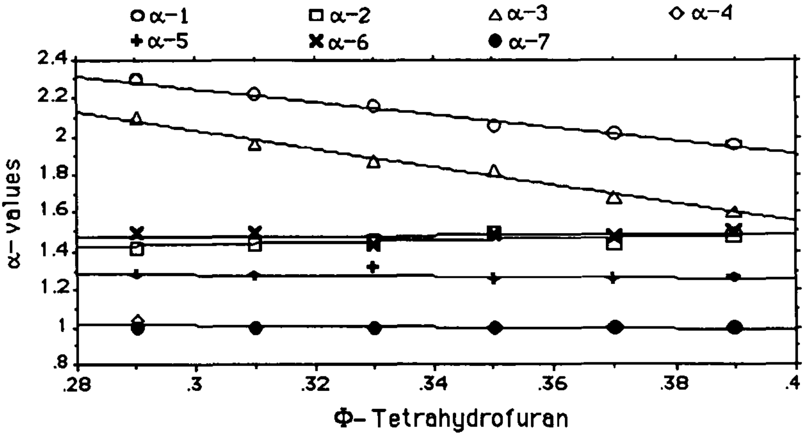


Figure 3. The effect of Φ-tetrahydrofuran on separation factor (α) (α-1=k′ of compound 2/k′ of compound 1, α-2=k′3/k′2, α-3=k′4/k′3, α-4=k′6/k′4, α-5=k′5/k′6, α-6=k′7/k′5, α-7=k′8/k′7; compounds see Fig. 1).

decreasing the volume ratio for dioxan. The separation between ostruthol and imperatorin decreased such that the a value approached 1.0. The other a values for compounds 2 and 3, 4 and 5, as well as 7 and 8, remained constant or only slightly increased when Φ decreased.

Figure 3 shows another type of behaviour for a values when plotted against Φ. The separation factor between the first three eluting compounds (1,2,3) strongly increased along with decreasing Φ values whereas a remained nearly constant for the other furocoumarins. Columbianadin and isoimperatorin were not separated at all: the a value remained at 1.0 throughout the change in Φ.

The other organic solvents, acetonitrile, methanol, ethanol and n-propanol, showed similar behaviour of the selectivity to dioxan; there were changes in the retention order. Only the α-value between compounds 2 and 3 remained at 1.0 throughout the change in Φ-ethanol.

Figure 4 and Table 1 show the effect of the mobile phase on the retention order of the closely related furocoumarins. Isoimperatorin (7) was eluted before columbianadin (8) in acetonitrile and dioxan. Tetrahydrofuran showed no selectivity to the separation of these compounds. In other solvents the retention order was reversed. The retention order of compounds 4, 5 and 6 changed considerably in different organic solvents. When the methanol was changed to ethanol and finally to n-propanol the elution order of compounds 2 and 3 was gradually reversed.

The retention (k') of furocoumarins, is listed in Table 1 for one concentration of organic solvent, giving approximately the same retention for the last eluting peak.

The retention behaviour of the furocoumarins was studied with reference to the molecular connectivity. Valence and connectivity indices, including path, cluster and path/cluster

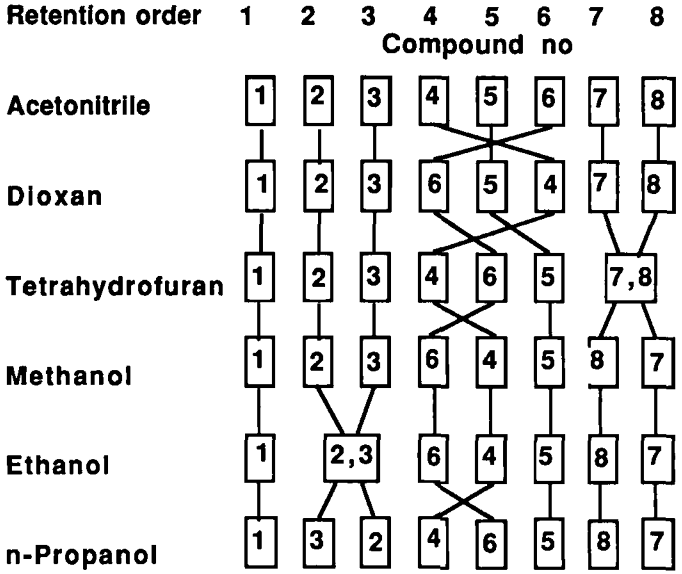


Figure 4. The retention order of eight furocoumarins in six different organic solvent/water mixtures presented in Table 1; Spherisorb ODS II as the stationary phase

Table 1 Capacity factor, k′ of the furocoumarins in six different organic solvent/water mixtures.

Compound no	ACN 38%	DIOXAN 41%	THF 29%
1	1.10	1.10	2.13
2	4.75	3.26	4.90
3	6.89	5.04	6.94
4.	11.83	13.30	14.62
5	12.77	12.14	19.38
6	16.29	11.62	15.16
7	27.30	25.16	29.00
8.	29.02	29.84	29.00

Table 1 (cont.)

Compound no	MeOH 54%	EtOH 42%	n-PrOH 25%
1.	1.94	2.20	2.37
2.	5.74	7.25	7.06
3.	7.28	7.25	6.06
4.	15.90	13.09	7.33
5.	19.25	15.51	14.82
6.	14.42	9.74	11.76
7.	30.69	35.00	35.82
8.	27.90	26.74	25.49

types, were calculated to the sixth order - totalling 28 different index values for each compound. When *ln k′* was plotted against each of these indices, the correlation was not generally high. Two and three-variable combinations of the indices and squares were also run, but the correlation showed no improvement.

High correlations were nevertheless obtained for certain structures, as demonstrated for group I (compounds 1, 3, 6 and 7) and group II (compounds 2, 3, 4, 5 and 8) in Figure 5 and Table 2.

The low order indices ($^0\chi^v$ and $^2\chi^v$) were chosen for group I, whereas the path/cluster type of the fifth order valence level index ($^5\chi_{pc}$) best described the retention of the furocoumarins in group *II*. Compound 3 ((+)-oxypeucedanin) was included in both groups since none of the calculated regressions showed any deviation from the other compounds in either groups.

(+)-Oxypeucedanin can also be regarded as an intermediate compound when two features of the structure are studied: the number of carbon atoms and the number of angelic acid units.

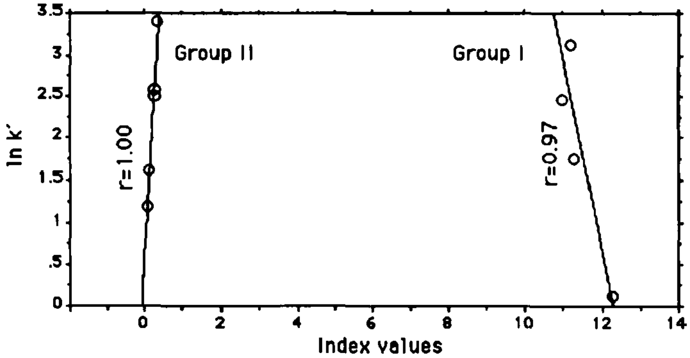


Figure. 5. ln (k') in dioxan-water for closely related furocoumarins
(Group I: compounds 1,3,6,7; Group II: compounds 2,3,4,5,8) plotted against the zero order valence level index for group I and against five order valence level path/cluster index for Group II.

Table 2. Molecular connectivity indices best describing the retention of the closely related furocoumarins (compounds see Fig. 1).

Compound no	Group	$^0\chi^v$	$^2\chi^v$	$^5\chi_{pc}$
1.	I	12.200	5.685	1.334
2.	II	8.520	3.408	0.951
3.	I,II	11.712	5.323	1.173
4.	II	17.480	7.437	2.063
5.	II	16.150	7.035	1.919
6.	I	11.300	4.810	0.974
7.	I	11.300	4.797	1.022
8.	II	14.210	6.490	2.517

As can be seen from Figure 5 and Table 2, the slopes of the regression equations are different for compounds in group I and II which may possibly indicate a different retention mechanism for these groups.

Throughout the Φ change, the correlation between ln k′of compounds in group I and the low order indices remained at the same level as for the lowest organic solvent concentration. When ln k′ was plotted against the path/cluster type of the fifth order valence level index for compounds in group II, an increase in Φ produced a decrease in the correlation. On the average, the coefficient of correlation changed linearly from 0.95 to 0.84 as the amount of organic solvent increased from the lowest to the highest concentration. The correlation obtained with high Φ values could not be improved by including other indices.

A feature typical of the compounds in group I is that the number of carbon atoms is the same. In group II the number of angelic acid units varies. Path/cluster type indices better take into account the branched parts of the molecules than the path type indices do (11) as in this case can be demonstrated for the compounds in group II.

Utilization of the molecular connectivity indices in predicting the retention of the closely related furocoumarins in different mobile phases was tested using the regression equations obtained from the data presented in Table 2. As can be seen from Figure 6 the retention equations gave a good correlation ($r=0.91$, $n=48$; $P<0.001$) between the predicted and observed k′ values. The correlation was especially high when the k′values lay below 10.

The present study shows that the separation factor (α) and retention order of the closely related furocoumarins can be

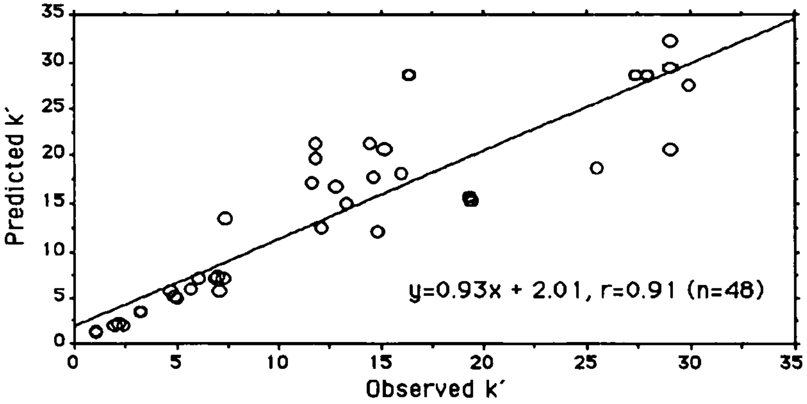


Figure 6. Plot of the observed and predicted capacity factors (k′) for the closely related furocoumarins in *Peucedanum palustre* .

The predicted values were calculated from equations derived from Table 2 and data obtained from Table 3.

Table 3. Regression coefficients for equations of the type $\ln k' = A\chi + B$ in six organic solvents (ACN, acetonitrile; THF, tetrahydrofuran; MeOH, methanol; EtOH, ethanol; n-PrOH, n-propanol).

Solvent	ACN		DIOXAN		THF	
Group	I	II	I	II	I	II
A	-3.43	1.10	-3.04	1.33	-2.55	1.10
B	42.10	0.71	37.24	-0.05	31.85	0.61
r	0.99	0.97	0.97	1.00	0.97	0.98
index χ	$^0\chi^v$	$^5\chi_{pc}$	$^0\chi^v$	$^5\chi_{pc}$	$^0\chi^v$	$^5\chi_{pc}$

Solvent	MeOH		EtOH		n-PrOH	
Group	I	II	I	II	I	II
A	-2.64	1.01	-2.33	0.82	-2.45	0.75
B	32.92	0.81	14.16	1.10	14.78	1.05
r	0.97	0.98	0.88	0.97	0.92	0.79
index χ	$^0\chi^v$	$^5\chi_{pc}$	$^2\chi^v$	$^5\chi_{pc}$	$^2\chi^v$	$^5\chi_{pc}$

influenced considerably by a change in Φ and the mobile phase. The retention can be predicted with great accuracy for these furocoumarins in several mobile phases, even if the compounds contain several oxygen atoms, by using molecular connectivity indices. This kind of approach can also be used as a diagnostic tool for closely related compounds: certain changes in α may mean predictable changes in the structure of a solute. The study encourages a more extensive investigation of the retention mechanism and the use of molecular connectivity in the HPLC analyses of coumarin type compounds.

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References

(1) Vuorela, H., J. Ethnopharmacol., submitted for publication.
(2) Vuorela, H., Törnqvist, K., Sticher, O., Hiltunen, R., Acta Endocrinologica, submitted for publication.
(3) Vuorela, H., Erdelmeier, C.A.J., Nyiredy, Sz., Dallenbach-Tölke, K., Anklin, C., Hiltunen, R., Sticher, O., Planta Medica, in press.
(4) Vuorela, H., Dallenbach-Tölke, K., Sticher, O., Hiltunen, R., J. Planar Chromatogr., **1**, 123,1988
(5) Vuorela, H., Dallenbach-Tölke, K., Nyiredy, Sz., Hiltunen, R., Sticher, O., Planta Medica, in press.
(6) Kaiser R.E., Oelrich E., Optimization in HPLC, Dr. Alfred Hüthig Verlag, Heidelberg-Basel-New York, 1981.
(7) Lehtonen P., Chromatographia **19**, 316, 1984.
(8) Wells M.J.M., Clark C.R., Patterson R.M., Anal. Chem., **58**, 1625, 1986.

(9) Burda J., Kuras M., Kriz J., Vodicka L., Z., Anal. Chem., **321**, 549, 1985.

(10) Randic M., J. Am. Chem. Soc., **97**, 6609, 1975.

(11) Kier L.B., Hall L.H., Molecular Connectivity in Chemistry and Drug Research, Academic Press, Inc., London 1976.

(12) Lehtonen P., J. Chromatogr., **314,** 141, 1984.

(13) Lehtonen P. Behaviour of Amine Derivatives in Reversed-phase Liguid Chromatography with reference to Molecular Structure, Academic Dissertation, University of Helsinki, Helsinki 1987.

(14) SAS Institute, Inc., SAS User's Guide. Statistics, SAS Institute, Inc., Cary, NC., 1982.