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Letter to the Editor

High-performance liquid chromatographic determination of urocanic acid in stripped human stratum corneum

Sir,

Recently, a new high-performance liquid chromatographic (HPLC) method for the determination of urocanic acid in human skin was published [1]. In spite of the lower sample weight (1—2 mg) compared with determinations in plantar callus [2], which require about 50 mg, convenient sampling on distinct body regions is still restricted. The method of choice might be adhesive tape stripping, which has been used in paper chromatography after alkaline extraction of urocanic acid [3—5]. Unfortunately, very large areas of skin must be stripped and extracted in order to obtain sufficient material for analysis. The combination of HPLC and stripping reduces the size of the sample to 3 cm^2, which results in a much more convenient sampling procedure for patients and volunteers.

The method was tested in ten volunteers (five males, five females) using Tesafilm (Beiersdorf, Hamburg, F.R.G.). An area of about 6 cm^2 was demarcated with Leukosilk (Beiersdorf) and twenty strips were successively taken from the same location (back). A glossy shine on the skin (reaching the statum granulosum) was recognized between the eleventh and fourteenth strips, depending on the subject. No feeling of pain, but only a slight burning sensation, was mentioned by the volunteers. Only moderate reddening was observed.

After cutting off 3-cm^2 pieces, they were extracted with 0.9 ml of 0.1 *M* potassium hydroxide solution in screw-capped vials by vortexing for 2 min. Then the samples were rotated head-over at 60 rpm in a rotator (Heidolph, Schwabach, F.R.G,.) for 15 min. Subsequently, 0.1 ml of orthophosphoric acid (0.66 mol/l) was added and the samples were mixed again. After removing the adhesive tape with forceps, 50 μl were injected into the chromatograph. The chromatographic conditions were as described previously [1]. The detector was operated at 264 nm.

The results are presented in Table I. The extraction efficiency was tested by adding 500 ng of authentic urocanic acid to a pre-extracted specimen; 491 ng were found (98.2% recovery). Blank strips contained no urocanic acid.

For recovery studies, amounts of 250, 500 and 750 ng of urocanic acid were

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TABLE I

UROCANIC ACID CONTENT (ng/cm²) IN DIFFERENT LAYERS OF STRIPPED HUMAN STRATUM CORNEUM

V = volunteer, m = male; f = female.

No. of strip	V_1 (m)	V_2 (f)	V_3 (m)	V_4 (f)	V_5 (f)	V_6 (m)	V_7 (m)	V_8 (f)	V_9 (f)	V_{10} (m)
1	170	213	93	120	283	543	162	393	784	165
2	237	437	70	156	334	395	156	272	423	136
3	70	507	83	167	347	394	250	426	348	198
4	333	550	177	146	330	340	180	573	317	227
5	260	610	140	143	420	310	278	590	290	291
6	294	533	237	157	370	350	184	542	328	350
7	307	577	217	206	497	264	208	450	236	276
8	297	437	257	184	343	305	194	503	277	307
9	273	407	260	133	403	238	212	432	246	258
10	240	320	190	186	353	265	246	423	170	216
11	207	340	177	187	340	233	202	327	161	224
12	230	220	93	186	384	212	144	140	78	174
13	137	173	117	173	227	175	126	260	77	132
14	77	107	97	113	230	173	94	210	45	141
15	50	70	54	163	200	110	53	188	53	98
16	30	50	44	140	134	110	56	130	17	76
17	33	77	33	87	108	72	44	110	15	32
18	40	80	20	60	89	62	27	75	7	21
19	34	97	20	37	51	40	26	50	10	38
20	30	4	23	50	46	23	18	37	12	25
Total	3349	5809	2402	2764	5489	4614	2860	6131	3894	3385

added to pulled-off strips, which were cut off from a 15-cm² strips sampled at one location. A mean of 97.0 ± 3.2% was obtained. Within-assay (see sample 10, volunteer 1 in Table I), 240 ± 14 ng/cm², coefficient of variation, 5.6%; between-assay, 247 ± 20 ng/cm², coefficient of variation, 8.1% (n = 5).

The method described enables the analyst to determine urocanic acid at any region of the body. The rate of analysis is higher than that of paper chromatography. Concentration profiles depending on the number of strips, i.e., "thickness" of the stratum corneum, can be readily performed. Reported localized differences in urocanic acid content [6] can easily be checked. By summing the values in all the strips, the total amount of urocanic acid in the stratum corneum for any body region can be calculated. Further, the influence of light on the (*E*)- to (*Z*)-isomerization of urocanic acid in different layers of the stratum corneum can be studied.

The method presented is currently being used to elucidate the possible role of urocanic acid in the etiology of polymorphous light eruption.

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