ELSEVIER

Journal of Chromatography B, 750 (2001) 71–80

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Analysis of histidine and urocanic acid isomers by reversed-phase high-performance liquid chromatography

Klaus Hermann*, Dietrich Abeck

Klinik und Poliklinik für Dermatologie und Allergologie am Biederstein, Technische Universität München, Biedersteiner Strasse 2, Munich, Germany

Received 12 November 1999; received in revised form 21 July 2000; accepted 24 July 2000

Abstract

The qualitative separation performance of a C_{18}, C_8 and C_4 reversed-phase column was investigated for the separation of histidine and its metabolites histamine, 1-methyihistamine and *trans*- and *cis*-urocanic acid. *Trans*- and *cis*-urocanic acid were baseline separated from their precursor histidine on all three columns using isocratic elution with a mobile phase composed of 0.01 *M* aqueous TEAP pH 3.0 and acetonitrile at a ratio of 98:2 (v/v). However, histidine was not separated from histamine and 1-methyihistamine. Selecting the C_8 column and introducing 0.005 *M* of the ion pairing reagent 1-octanesulfonic acid sodium salt into the aqueous solution and acetonitrile at a ratio of 90:10 (v/v), significantly improved the separation. The separation was also followed by a change in the retention times and the order of elution. The sequence of elution was histidine, *cis*-urocanic acid, *trans*-urocanic acid, histamine and 1-methylhistamine with retention times of 5.58±0.07, 7.03±0.15, 7.92±0.18, 18.77±0.24 and 20.79±0.21 min (mean±SD; $n=5$). The separation on the C_8 column in the presence of the ion-pairing reagent was further improved with gradient elution that resulted in a reduction in the retention times and elution volumes of histamine and 1-methylhistamine. The detection limits of histidine and *trans*-urocanic acid at a wavelength of 210 nm and an injection volume of 0.05 ml were 5×10^{-8} mol l^{-1} ($n=3$). The kinetic of the in-vitro conversion of *trans*- into the *cis*-isomer after UV irradiation was depending on the time of exposure and the energy of the light source. UVB light induced a significantly faster conversion than UVA light. TUCA and cUCA samples kept at −25°C were stable for up to 50 weeks. Samples, eluted from human skin showed various concentrations of histidine and *trans*- and *cis*-urocanic acid with an average of $1.69\pm0.33\times10^{-5}$ mol l^{-1}, $1.17\pm0.43\times10^{-5}$ mol l^{-1} and $1.67\pm0.33\times10^{-5}$ mol l^{-1}, respectively ($n=8$). © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Histidine; Urocanic acid

1. Introduction

Urocanic acid (UCA), an imidazole-acrylic acid derivative is an endogenous component of the stratum corneum of the human skin where it is present in high concentration [1]. It is synthesized in vivo from the amino acid histidine (Hid) by the enzyme histidase, a histidine-ammonia-lyase (EC 4.3.1.3) [2]. UV irradiation of the skin induces a photo-isomerization of the naturally occurring *trans*-isomer (tUCA) into the *cis*-isomer (cUCA) [3]. The physiological significance of UCA in the human skin is its important role as a natural sunscreen with photoprotecting activity against UV damage to DNA [4,5]. In addition, cUCA has been linked to UV-

*Corresponding author. Tel.: +49-89-4140-3207; fax: +49-89-4140-3323.

0378-4347/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(00)00416-3

induced immunological alterations with systemic immune suppression that may play a critical role in skin cancer development [6,7]. Several studies have shown that skin cancer has increased significantly in the last few years. Reasons for this phenomenon are the indolent exaggerating sun bathing habits, the increasing use of artificial UV irradiation in sun tanning beds and the reduction of the ozone layer with the subsequent augmented terrestrial UV irradiation [8–10]. Therefore, the measurement of UCA in the human skin as a marker for the degree of UV irradiation might be of clinical importance. This study focused on the methodological evaluation of high-performance liquid chromatography (HPLC) for the qualitative and quantitative determination of tUCA and cUCA, their precursor Hid and other metabolites of Hid such as histamine (His) and 1-methyihistamine (MH). Likewise, the stability and the in-vitro conversion of tUCA into cUCA after UV irradiation were studied. The method was used for the characterization and measurement of Hid, tUCA and cUCA in human skin eluates.

2. Materials and methods

2.1. High performance liquid chromatography (HPLC)

HPLC separations were performed on an ERC HPLC gradient equipment which was composed of a ERC-3512 degasser, 2 ERC-64 HPLC pumps, a SOMA S-3702 UV/VIS detector and a manual Rheodyne injection valve with a 0.1 ml sample loop. A Borwin HPLC software was used for data acquisition and controlling the HPLC system. All solvents used were filtered through a RC-55 membrane filter (Schleicher and Schüll, Dassel, Germany) with a pore size of 0.45 μm prior to use. Three reversed-phase columns, a C_{18}, C_8 and C_4 with 250 mm×4.6 mm and a pore size of 5 μm were investigated. A mobile phase composed of a mixture of solvent A, 0.01 *M* triethyl-ammoniumphosphate (TEAP), adjusted to pH 3.0 with concentrated orthophosphoric acid, and B, acetonitrile (ACN) at a ratio of 98:2 (v/v) was used for the three columns. Separations were also performed on the C_8 column using solvent A with the addition of 0.005 *M* 1-octanesulfonic acid sodium salt (OSA) as an ion pairing reagent and solvent B, ACN at a ratio of 90:10 (v/v). In addition, gradient elution was performed on the C_8 column with solvent A, 0.01 *M* TEAP pH 3.0 with 0.005 *M* OSA and solvent B, ACN. Initial conditions: 10% B for 5 min followed by a linear increase in B to 18% within 1 min. The column was then developed with 18% B. All separations were carried out at room temperature (22°C) with a flow-rate of 1.0 ml min^{-1} and were monitored with UV detection at a wavelength of 210 nm for Hid, His, MH, tUCA and cUCA. Likewise, tUCA and cUCA were also monitored at 267 nm. In all instances, 0.05 ml standards or samples were injected.

2.2. Preparation of standards

His, MH, Hid and tUCA were dissolved in 0.01 N HCl at a concentration of 10^{-2} mol l^{-1} in 1.5 ml plastic tubes (Eppendorf, Hamburg, Germany). These stock solutions were diluted with 0.001 N HCl to 10^{-5} or 10^{-6} mol l^{-1} for the qualitative analysis. The Hid and tUCA stock solution was further diluted with 0.001 N HCl to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 5×10^{-8}, 2.5×10^{-8}, 10^{-8} and 10^{-9} mol l^{-1} for the calibration curves. The 10^{-2} mol l^{-1} tUCA stock solution had to be slightly heated in order to get the tUCA in solution. Special care has been taken to the tUCA standards avoiding UV light by wrapping the tubes with aluminum foil. One set of 0.3 ml tUCA standards in 1.5 ml Eppendorf tubes ranging from 10^{-3} to 10^{-8} mol l^{-1} was analyzed by HPLC. Another set of 0.3 ml tUCA standards in 1.5 ml Eppendorf tubes ranging from 10^{-3} to 10^{-8} mol l^{-1} was irradiated with a sun-simulator (Dermalight Vario II, Dr. Hoenle, Martinsried, Germany) to generate cUCA. The samples were exposed to the light source providing UVB, UVA and visible light at a distance of 120 cm for 60 mm resembling 0.012 J cm^{-2} min^{-1} UVB and 0.102 J cm^{-2} min^{-1} UVA. Standards were run in duplicates and unknowns were calculated from the calibration curve.

2.3. In vitro conversion of tUCA into cUCA

A 10^{-4} mol l^{-1} tUCA solution, dissolved in 0.001 N HC1 was pipetted into 1.5 ml Eppendorf tubes. Each tube with a volume of 0.3 ml was irradiated

with the sun-simulator for 0, 1, 2, 5, 10, 20, 30, 60 and 120 mm within 120 cm distance to the light source. The tubes were removed from the UV light source at the different times and covered with an aluminum foil and kept frozen at −25°C prior to HPLC analysis.

2.4. Stability of UCA samples

TUCA samples at a concentration of 10^{-4} mol l^{-1} (0.3 ml in 1.5 ml Eppendorf tubes) were exposed to the sun-simulator at a distance of 20 cm for 0, 1, 2, 5, 10, 20, 30 and 60 min to generate cUCA. The irradiated samples were wrapped with aluminum foil and kept for 1 day in the refrigerator and then analyzed on HPLC. The same samples were further stored at −25°C for 4, 11 and 50 weeks and analyzed again on HPLC.

2.5. Preparation of skin samples

Human skin samples were taken in summer time from eight healthy Caucasian volunteers (two males and six females, mean age 33±6 years) without any differences in skin coloration. UCA was extracted from the skin with the elution method described by Stab et al. [11]. A glass funnel with a surface area of 14.52 cm was tightly pressed on to the skin of the inner side of the left forearm. The funnel was filled with 3.0 ml of a mixture of ethanol and water (1:1, v/v) and the extraction was carried out for 3 min by gently agitating the solvent in rotary movements. The extracts were collected in 15 ml plastic tubes and dried in vacuum centrifuge. The dried samples were reconstituted in 0.5 ml 0.001 N HCl, spun for 3 min at room temperature and 13 000 *g* prior to injection. Results are expressed as mean±SEM in 10^{-5} mol l^{-1}.

2.6. Chemicals

All chemicals used were of analytical grade. Hid, His and tUCA were purchased from Sigma (Sigma, Deisenhofen, Germany). MH was obtained from Calbiochem (Calbiochem, Frankfurt, Germany) and OSA, TEA and concentrated ortho-phosphoric acid came from Fluka (Fluka, Germany). HPLC solvents and 0.1 *M* HCl were delivered by Merck (Merck, Darmstadt, Germany).

3. Results

3.1. Separation of standards

Three different reversed-phase columns, a C_{18}, C_8 and C_4 were investigated for the qualitative separation of Hid from His, MH, tUCA and cUCA. A baseline separation of tUCA from cUCA was achieved isocratically with an aqueous solution of 0.01 *M* TEAP pH 3.0 and ACN at a ratio of 98:2 on all three columns. The retention times for tUCA and cUCA were 5.19±0.05 and 8.15±0.10 min on the C_{18} column ($n=6$), 5.03±0.03 and 7.88±0.06 min ($n=4$) on the C_8 column and 4.38±0.02 and 5.29±0.04 min ($n=3$) on the C_4 column. Likewise, Hid, the precursor of UCA was separated from tUCA and cUCA. However, Hid was not separated from its metabolites His and MH (Table 1).

The C_8 column was further studied for the separation of Hid, tUCA, cUCA, His and MH. In the absence of OSA a baseline separation between Hid, tUCA and cUCA was accomplished whereas His and MH coeluted (Fig. 1, top panel). The separation was significantly improved by introducing OSA as an ion-pairing reagent. A baseline separation between Hid, His, MH, tUCA and cUCA was achieved isocratically with the addition of 0.005 *M* OSA to

Table 1
Retention times of histidine, histamine, 1-methylhistamine, tUCA and cUCA on a C_{18}, C_8 and C_4 column in the absence of the ion pairing reagent OSA at a wavelength of 210 nm[a]

	L-histidine	histamine	1-methylhistamine	tUCA	cUCA
C_{18} column	2.03 +/− 0.02	1.80 +/− 0.02	1.85 +/− 0.05	5.19 +/− 0.05	8.15 +/− 0.10
C_8 column	2.83 +/− 0.01	2.54 +/− 0.01	2.58 +/− 0.03	5.03 +/− 0.03	7.88 +/− 0.06
C_4 column	3.19 +/− 0.01	3.51 +/− 0.12	3.39 +/− 0.43	4.38 +/− 0.02	5.29 +/− 0.04

[a] A baseline separation was achieved for Hid, tUCA and cUCA. However, Hid was not separated from its metabolites His and MH.

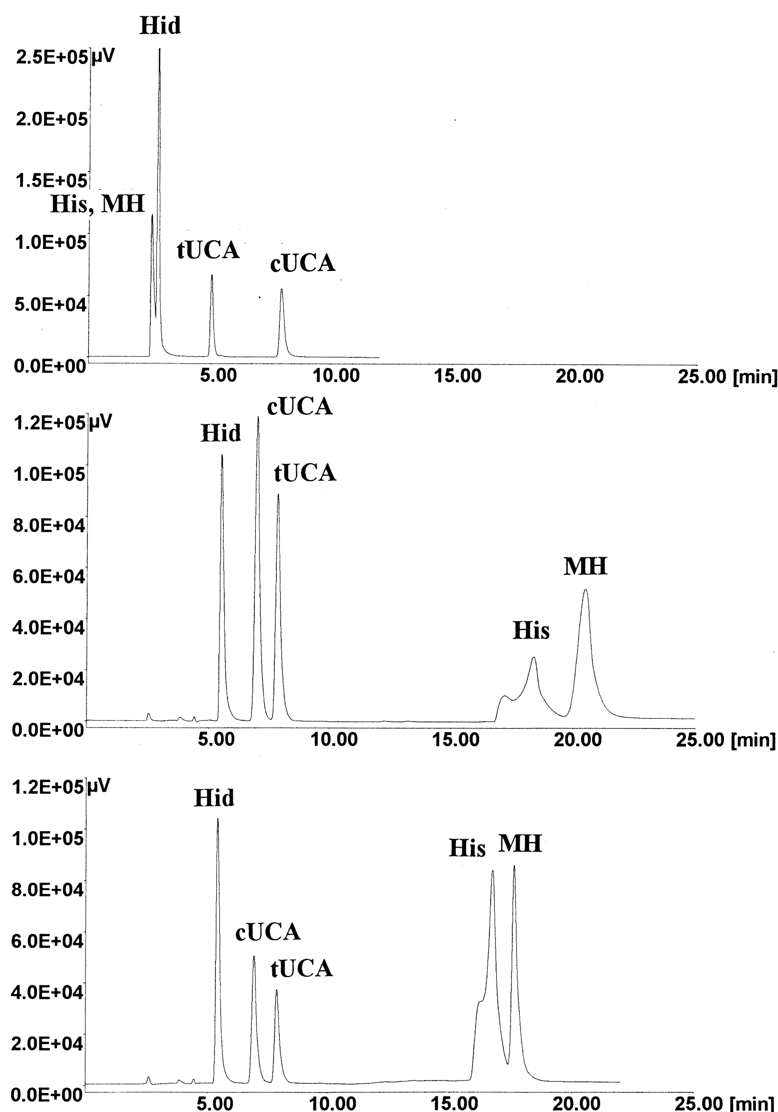

Fig. 1. HPLC separation of Hid, His, MH, tUCA and cUCA on the reversed-phase C_8 column. Top panel: A baseline separation between Hid, tUCA and cUCA was accomplished isocratically in the absence of the ion-pairing reagent OSA. His and MH coeluted and were incompletely separated from Hid. Middle panel: Hid, tUCA, cUCA, His and MH were separated isocratically in the presence of the ion-pairing reagent OSA. Bottom panel: The separation was significantly improved with gradient elution. Note the reduction in the retention times, improved peak shape and elution volume of the His and MH peaks.

the aqueous 0.01 *M* TEAP solution and ACN at a ratio of 90:10. The compounds eluted in the sequence of Hid, cUCA, tUCA, His and MH with retention times of 5.54±0.04 min (n=5), 6.98±0.09 min (n=5), 7.92±0.10 min (n=5), 18.77±0.34 min (n=5) and 20.72±0.25 min (n=5) (Fig. 1, middle panel). The elution sequence of tUCA and cUCA in the absence or presence of OAS was different. TUCA eluted before cUCA in the absence of OSA on the C_8 column whereas cUCA eluted before tUCA in the presence of OSA. The separation was further refined by using gradient elution which

reduced the elution volume and retention times of His to 16.74 min and MH to 17.63 min (Fig. 1 bottom panel).

3.2. In vitro conversion of tUCA into cUCA

TUCA samples that have been irradiated with a sun-simulator showed a time-dependent decrease in tUCA that was accompanied with a time-dependent increase in cUCA (Fig. 2, top panel). The kinetic of the conversion of tUCA into cUCA was also dependent on the energy of the UV irradiation. The conversion with UVB light was significantly faster as compared to UVA light (Fig. 2 bottom panel).

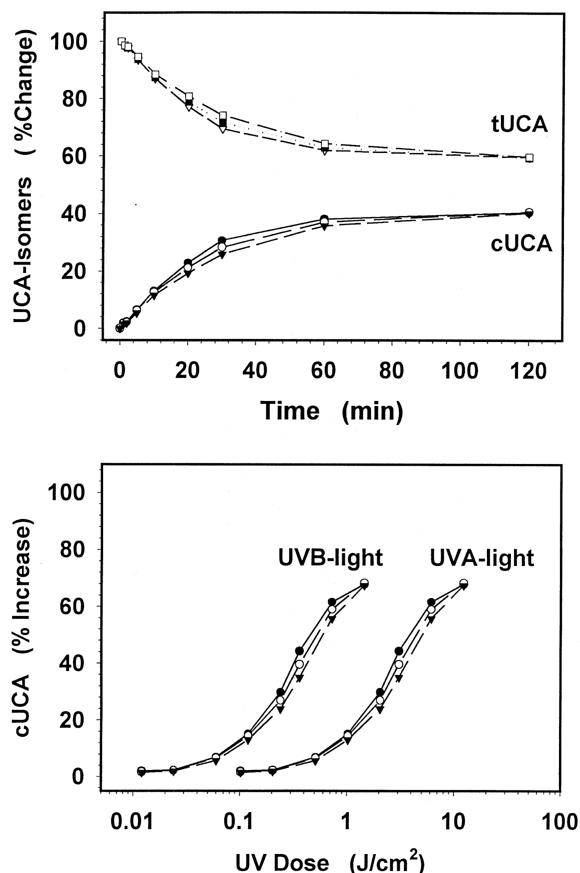

Fig. 2. In-vitro conversion of tUCA into cUCA after irradiation with a sun-simulator. Top panel: The kinetic of the conversion was closely related to the duration of the exposure with a time-dependent decrease in tUCA that was accompanied by an increase in cUCA. Bottom panel: The conversion was also associated with the energy of the UV light. The conversion after irradiation with UVB light was significantly faster as compared to UVA light.

3.3. Calibration curves of Hid and UCA

Calibration curves with Hid and tUCA standards on the C_8 column in the presence or absence of OSA were linear in the range between 10^{-4} to 10^{-8} mol l^{-1} with a correlation coefficient of $r^2=0.997$ and $r^2=0.999$, respectively. The detection limits of Hid and tUCA standards at a wavelength of 210 nm were 5×10^{-8} mol l^{-1}. The detection limit of tUCA was slightly improved to 2.5×10^{-8} mol l^{-1} at a wavelength of 267 nm (Fig. 3).

3.4. Stability of UCA-isomers

UCA samples irradiated with the sun-simulator were stable over a long period of time. No significant differences in the concentration of tUCA or cUCA were found between samples that were analyzed within 1 day after UV irradiation or samples that were stored at −25°C for 4, 11 or 50 weeks. The table also shows the high reproducibility of the HPLC procedure with a standard deviation of <1 for all samples tested (Table 2).

3.5. Determination of Hid, tUCA and cUCA in skin elutes

Reconstituted skin samples were separated isocratically on the C_8 column in the absence or presence of OSA.

In the absence of OSA, Hid, tUCA and cUCA were identified with UV detection at a wavelength of 210 nm. In addition, several other compounds were detected at 210 nm (Fig. 4, top panel). All these peaks including the Hid peak vanished when the separation was carried out at 267 nm (Fig. 4, middle panel). Monitoring the separation at 267 nm also increased the detection signal for cUCA and tUCA by 40 to 50%. A sample that was spiked with Hid, tUCA and cUCA showed identical retention times for all three compounds (Fig. 4, bottom panel).

In the presence of OSA, Hid, tUCA and cUCA were also identified at 210 nm. The chromatogram of two samples is shown in Fig. 5 as an example. It shows a sample with low concentrations of Hid,

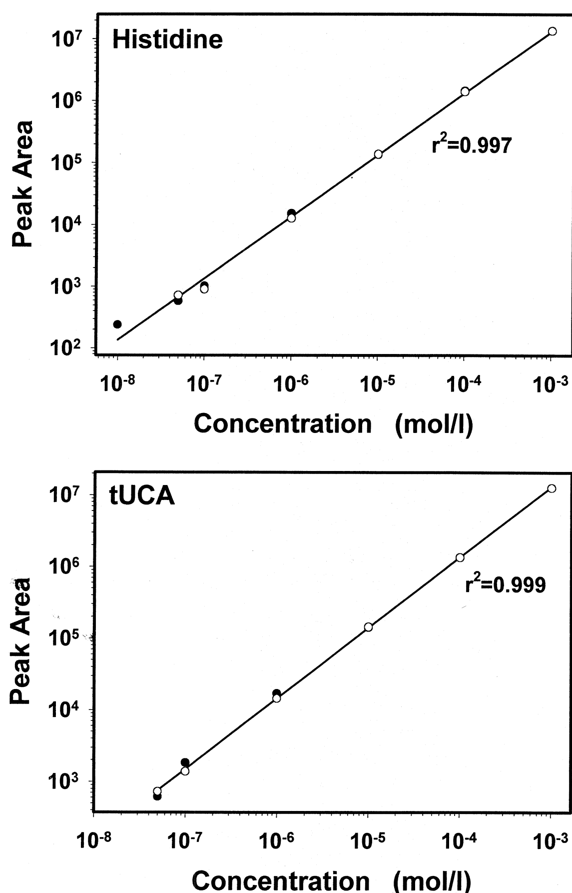

Fig. 3. Calibration curves of Hid and tUCA standards on a reversed-phase C_8 column in the presence of OSA at 210 nm. The calibration curves of Hid and tUCA were linear between 10^{-4} to 10^{-8} mol l^{-1} with a correlation coefficient of r^2=0.997 and r^2=0.999, respectively. The detection limits of Hid and tUCA standards were 5×10^{-8} mol l^{-1}.

cUCA and tUCA (Fig. 5, top panel) and a sample with high concentrations of Hid, cUCA and tUCA (Fig. 5, middle panel). Spiking the sample with the low Hid, cUCA and tUCA concentrations with the corresponding standards showed a coelution of the standards with endogenous compounds (Fig. 5, bottom panel).

Various concentrations of Hid, cUCA and tUCA were found in the skin samples. Predominantly, cUCA was discovered in the skin elutes with an overall ratio of 62.94±7.07% cUCA to 37.02±7.04% tUCA (n=8). The concentrations of Hid ranged from 0.60 to 3.00×10^{-5} mol l^{-1} with an average concentration of $1.69\pm0.33\times10^{-5}$ mol l^{-1} (n=8). An average concentration of $1.17\pm0.43\times10^{-5}$ mol l^{-1}, ranging from 0.41 to 4.05×10^{-5} mol l^{-1} was found for tUCA (n=8). The concentrations of cUCA ranged from 0.78 to 3.64×10^{-5} mol l^{-1} with an average of $1.67\pm0.33\times10^{-5}$ mol l^{-1} (n=8). His or MH was not detectable in all of the skin samples tested.

4. Discussion

Several HPLC procedures have been described for the qualitative and quantitative determination of tUCA and cUCA in human skin using polar or reversed-phase packing materials with aqueous buffers and acetonitrile or isopropanol as organic modifiers [12–15]. These HPLC procedures were able of separating tUCA from cUCA but none of these publications mentioned or showed the separation of Hid from tUCA and cUCA. However, the capability of measuring Hid besides tUCA and cUCA is important. Since Hid is the precursor of UCA, the endogenous Hid concentrations may influence the UCA concentrations in the skin. In fact, it has been shown that the total amount of UCA in the skin is significantly higher after a Hid-rich diet as compared to a normal diet [16]. In this study we used a reversed-phase C_{18}, C_8 or C_4 column for the separation of tUCA and cUCA and their precursor Hid. Hid, tUCA and cUCA standards were well separated from each other on all three columns. However, His and MH, two other Hid metabolites were only partially separated from Hid. Hid, His, MH, tUCA and cUCA showed a low affinity to the matrix of the three columns due to their relative high polarity. Minor retention of all five compounds was only possible with a very low concentration of 2% ACN in the mobile phase.

The C_8 column was chosen for further investigations with or without the addition of the ion-pairing reagent OSA. OSA forms an ion pair with the positively charged analytes that leads to an increase in hydrophobicity. Under these conditions, the separations had to be carried out with 10% ACN instead of 2% in the absence of OSA. Introducing OSA improved the separation significantly. Under these conditions, Hid was not only separated from

Table 2
Stability of UV-irradiated UCA samples after storage for 1 day in the refrigerator or 4, 11 and 50 weeks at −25°C[a]

Time (min)	1 Day		4 Weeks		11 Weeks		50 Weeks		tUCA		cUCA	
	%tUCA	%cUCA	%tUCA	%cUCA	%tUCA	%cUCA	%tUCA	%cUCA	Mean	SD	Mean	SD
0	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
1	79.88	20.12	79.49	20.51	79.88	20.12	79.34	20.66	79.65	0.27	35.10	0.27
2	68.87	31.13	68.47	31.53	68.77	31.23	68.35	31.65	68.62	0.24	40.62	0.24
5	58.87	41.13	58.54	41.46	58.90	41.10	58.45	41.55	58.69	0.23	45.58	0.23
10	56.62	43.38	56.55	43.45	56.88	43.12	56.43	43.57	56.62	0.19	46.66	0.19
20	56.13	43.87	56.08	43.92	56.35	43.65	55.95	44.05	56.13	0.17	46.91	0.17
30	56.11	43.89	56.10	43.90	56.32	43.68	56.07	43.93	56.15	0.11	46.90	0.11
60	56.29	43.71	56.31	43.69	56.59	43.41	57.49	42.51	56.67	0.56	46.49	0.56

[a] Samples were stable up to 50 weeks without any significant changes in the tUCA or cUCA concentrations. The results also show the high reproducibility of the HPLC procedure with a standard deviation of <1 for all samples tested.

tUCA and cUCA but also from His and MH. Likewise, a change in the retention times and the order of elution was noted. An even better separation between His and MH was achieved by gradient elution that improved the peak shape and the elution volume and led to a reduction in their retention times.

In-vitro conversion of tUCA into cUCA was performed with a sun-simulator providing UVB, UVA and visible light. The degree of the conversion was closely related to the time of exposure with an exponential decrease in tUCA that was accompanied by an exponential increase in cUCA. Likewise, the energy-rich UVB light induced a significantly faster conversion as compared to the UVA light with lower energy.

Calibration curves of Hid and tUCA standards performed on the C_8 column with or without OSA were linear over a wide range of concentrations from 10^{-4} mol l^{-1} to 5×10^{-8} mol l^{-1} at 210 nm with a correlation coefficient of $r^2=0.997$ and $r^2=0.999$, respectively. The detection limit of Hid and tUCA were 5×10^{-8} mol l^{-1} corresponding to approximately 350 pg on column. The sensitivity was even further improved by 40 to 50% down to 2.5×10^{-8} mol l^{-1} for tUCA when the separation was performed at 267 nm, the major absorbence maximum of UCA. However, the Hid peak vanished at 267 nm and the detection was only possible at 210 nm.

The stability of tUCA and cUCA was excellent over a long period of time when stored in the freezer at −25°C and light protected with aluminum foil. No significant changes in the tUCA or cUCA concentrations were found in samples analyzed one day after UV irradiation or samples stored for 4, 11 or 50 weeks. These results also showed the high reproducibility of the HPLC procedure with a standard deviation of <1 for all samples tested.

The C_8 column with or without OSA was used for the determination of Hid, tUCA and cUCA in human skin eluates. Various techniques for sampling UCA from the skin are described in the literature including scraping [16], punch biopsy [17] or elution with 0.1 *M* potassium hydroxide [12]. Since these are very invasive techniques we decided to extract Hid, and the UCA isomers from intact skin by the less invasive elution procedure with a mixture of 50% ethanol in water described by Stab et al. [11]. All of the different sampling techniques have never been thoroughly investigated with respect to extraction time, extraction volume or recovery. It is nearly impossible to design and perform an accurate experiment to account for these factors. Since Hid and UCA are located in the skin, topical application or addition to the extraction medium are far away from the real in-vivo condition. Likewise, the use of radioactive-labeled compounds for these types of experiments can be ruled out because of ethical reasons. The skin samples could be used without laborious purification or derivatization after reconstitution in diluted HCl. In all irradiated samples, Hid, tUCA and cUCA were found with prevailing amounts of the *cis*-isomer at a ratio of 7 to 3, respectively. The procedure was highly sensitive and capable of distinguishing even slight differences in the ratio between Hid, tUCA and cUCA. The

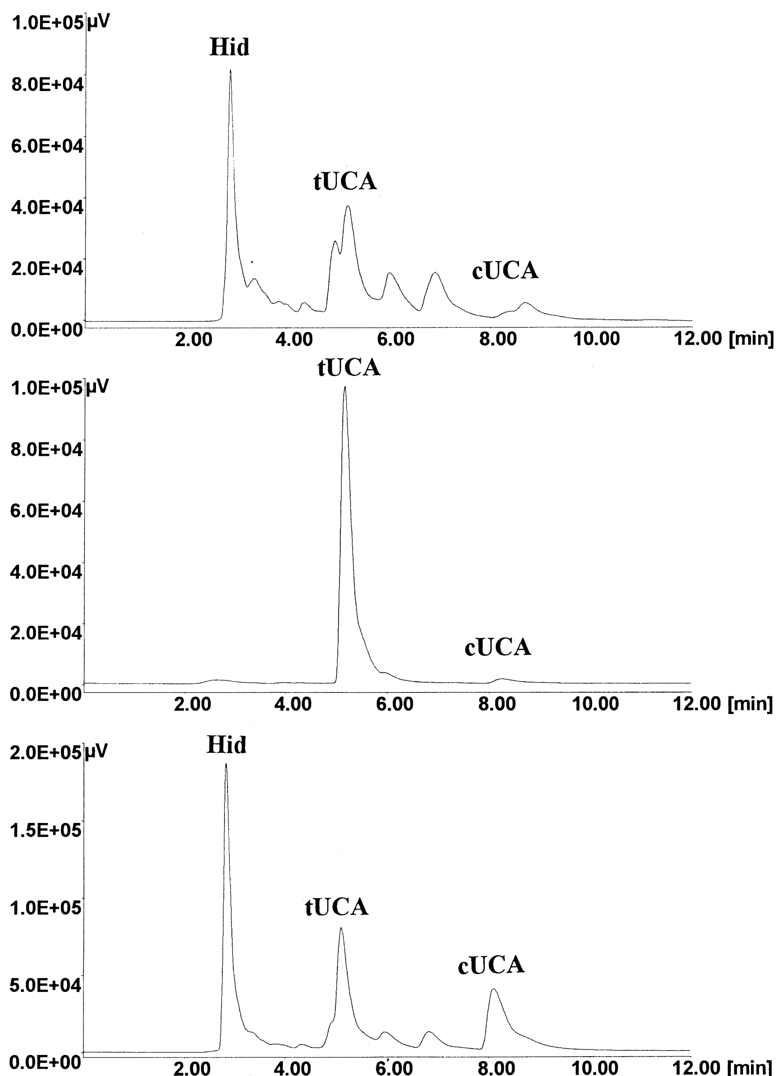

Fig. 4. Isocratic HPLC separation of Hid, tUCA and cUCA in a human skin sample on the reversed-phase C_8 column in the absence of the ion pairing reagent OSA. Top panel: Separation of a sample monitored at a wavelength of 210 nm with numerous other compounds. Middle panel: Separation of the same sample monitored at a wavelength of 267 nm where the Hid peak and other compounds vanished and only tUCA and cUCA were picked up. Bottom panel: Separation of the same sample monitored at 210 nm that was spiked with Hid, tUCA and cUCA.

identification was confirmed by spiking skin samples with authentic Hid, tUCA and cUCA and co-elution of the endogenous compounds with the standards. Various other compounds besides Hid, tUCA and cUCA were visible at 210 nm. At a wavelength of 267 nm, the majority of the peaks vanished including the Hid peak but leaving the tUCA and cUCA peak with even higher signals. Although the detection at 210 nm is not very specific it is very sensitive and reproducible. The capability of measuring Hid besides tUCA and cUCA is important since Hid is the precursor of UCA and the endogenous Hid concentrations affect the UCA concentrations in the skin [16]. Therefore, the HPLC separations were monitored at 210 nm, which allowed the simultaneous determination of Hid and the UCA isomers in

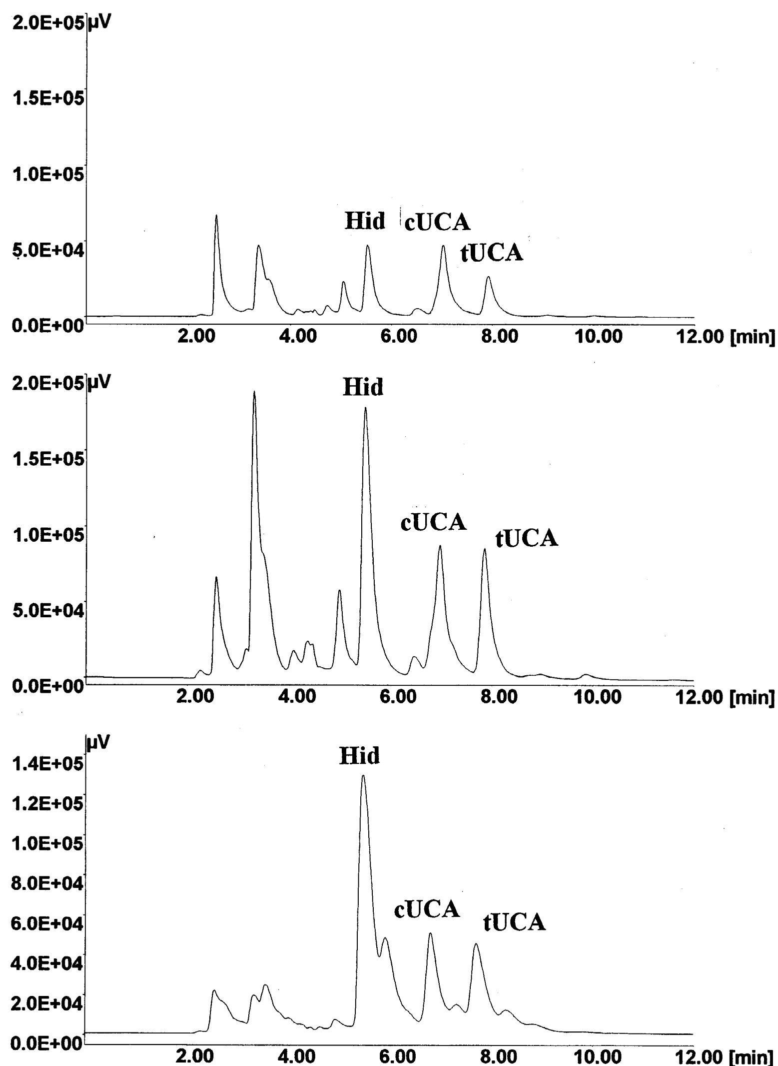

Fig. 5. Isocratic HPLC separation of Hid, cUCA and tUCA in human skin samples on the reversed-phase C_8 column in the presence of the ion pairing reagent OSA at a wavelength of 210 nm. Top panel: Skin sample with low concentrations of Hid, cUCA and tUCA. Middle panel: Skin sample with high concentrations of Hid, cUCA and tUCA. Bottom panel: Skin sample that was spiked with Hid, cUCA and tUCA.

a single run. Derivatization of Hid or UCA with *o*-phthaldialdehyde (OPA) to improve sensitivity and specificity was not possible since UCA did not react with OPA.

In summary: a simple, fast, reproducible and highly sensitive HPLC procedure for the qualitative and quantitative determination of Hid, tUCA and cUCA in human skin elutes is described. The separation can be accomplished on a C_8 column in a single run with or without the ion pairing reagent OSA within 10 min and a detection limit of 5×10^{-8} mol l^{-1}.

References

[1] M. Norval, T.J. Simpson, E. Bordshin, J. Crosby, Photodermatology 6 (1989) 142.

[2] H.P. Baden, M.A. Pathak, J. Invest. Dermatol. 48 (1967) 11.
[3] H. Morrison, Photodermatology 2 (1985) 158.
[4] A. Zenisek, J.A. Kral, I.M. Hais, Biochim. Biophys. Acta 18 (1955) 589.
[5] G. Kavanagh, J. Crosby, M. Norval, Br. J. Dermatol. 133 (1995) 728.
[6] F.P. Noonan, E.C. De Fabo, Immunol. Today 13 (1992) 250.
[7] F.P. Noonan, E.C. DeFabo, M.L. Kripke, Photochem. Photobiol. 34 (1981) 683.
[8] WHO Fact Sheet No 133, September 1996.
[9] B.A. Gilchrest, M.S. Eller, A.C. Geller, M. Yaar, The pathogenesis of melanoma induced by ultraviolet radiation, New Engl. J. Med. 340 (17) (1999) 1341.
[10] Environmental Council, American Academy of Dermatology, Am. Acad. Dermatol. 41 (1999) 81.
[11] F. Stäb, U. Hoppe, G. Sauermann, J. Invest. Dermatol. 102 (1995) 666A.
[12] C.T. Jansen, K. Lammintausta, P. Pasanen, K. Neuvonen, E. Varjonen, K. Kalimo, P. Äyräs, Acta Derm. Venereol. (Stockh.) 71 (1991) 143.
[13] W. Schwarz, K. Langer, A. Haag, J. Chromatogr. 310 (1984) 188.
[14] J.C. Caron, B. Martin, B. Shroot, J. Chromatogr. 230 (1982) 125.
[15] H. Morrison, D. Avnir, Th. Zarrella, J. Chromatogr. 183 (1980) 83.
[16] S.K. Reilly, E.C. De Fabo, Photochem. Photobiol. 53 (4) (1991) 431.
[17] L.J. Webber, E.W. Whang, E.C. De Fabo, Photochem. Photobiol. 66 (4) (1997) 484.