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Simple and sensitive high-performance liquid chromatography method for simultaneous determination of urinary free cortisol and 6β-hydroxycortisol in routine practice
For CYP 3A4 activity evaluation in basal conditions and after grapefruit juice intake

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Abstract

Cytochrome P450 3A4 activity displays a wide variability. The urinary 6β-hydroxycortisol to cortisol ratio, as a non-invasive assay, can be useful for its pretherapeutic characterization. We developed an HPLC–UV method preceded by liquid–liquid extraction for assessment of this ratio in clinical practice. Urine was collected on second void morning-spot sample. Percentage recoveries were high and reproducible. The 6β-hydroxycortisol to cortisol ratio ranged from 1.6 to 9.9 in 12 Caucasian healthy volunteers. It was reduced by 30 to 70% after ingestion of white grapefruit juice, a CYP3A4 inhibitor. Our method, simple, sensitive and accurate, could be helpful for determination of CYP 3A4 activity before oral chemotherapy, or for the monitoring of the use of grapefruit juice as a pharmacological modulator.

Keywords: Cortisol; 6β-Hydroxycortisol

1. Introduction

Cytochrome P450 3A4 (CYP3A4) is the most abundant form of cytochrome in humans. It is highly expressed in the liver, accounting there for approximately 30% of the total cytochrome content, but also in the small intestine, accounting for nearly 70% of the content [1]. It is responsible for the metabolism of the majority of drugs currently available [2]. Its expression varies widely, mainly due to exogenous factors. Thus, it can be induced or on the contrary, inhibited by numerous compounds, and as such, it is responsible for many drug–drug or drug–substance interactions [3]. A wide variety of exogenous substances inhibiting CYP3A4 activity have been identified. The most clinically relevant ones are cyclosporin A, erythromycin, indinavir, ketoconazole and grapefruit juice, which is the only substance known as a suicide mechanism-based inhibitor [4–6].

These interactions, due to enzyme induction as

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well as inhibition, can compromise efficacy or safety of a drug in clinical practice, especially if it has a low therapeutic index such as antineoplastic drugs. Thus, Irinotecan (CPT-11), docetaxel, paclitaxel, vincaalkaloïds and ZD 1839 metabolisms are known to exhibit significant variability that could interfere with both their efficacy and their tolerance [7–9].

Several in vivo indirect measures of CYP3A4 activity have been investigated, including the erythromycin breath test (ERMBT), midazolam plasma clearance and the 6β-hydroxycortisol/free cortisol ratio (6β-OHC/FC) in urine [10–12]. Measurement of urinary 6β-hydroxycortisol/free cortisol ratio simply requires a urine collection and is presently the only non-invasive assay of CYP3A4 activity. Although its use has been debated, this ratio is considered as the simplest and most practical method in the assessment of CYP3A4 activity induction and inhibition [13–15]. Two concurrent approaches have been developed: 24 h urine collection, and single morning spot urine sample. The first approach is time consuming and may be hampered by potential errors such as inadequate urine collection. For the second, excretion of 6β-OHC and FC show strong diurnal rhythms, but the two rhythms are so close that no significant variation of the urinary ratio of 6β-OHC to FC has been reported within the day [14,16]. The objective of the present study was to set up a simple and sensitive method of second void morning-spot urinary 6β-OHC/FC ratio measurement to determine CYP3A4 activity and investigate its usefulness in predicting the metabolism and interaction of certain antineoplastic drugs. Furthermore, we wanted to study the in vivo inactivation of CYP3A4 by white grapefruit juice (WGJ) so as to find ultimately the best scheme of administration for inducing significant and prolonged CYP3A4 inhibition. Even if HPLC methods have already been developed in that indication, they may present some difficulties because of the specific apparatus used such as a mass spectrometer [17–19], or extraction with cartridges [20,21]. They also often require different conditions for the detection of the two compounds [22,23].

Thus, we looked for a simple and rapid HPLC method for simultaneous detection of 6β-OHC and FC in urine, easily set up in every laboratory able to conduct basic extraction methods. Furthermore, our method is compatible with grapefruit juice ingestion avoiding interferences with urinary excreted compounds.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of HPLC or reagent-grade and were obtained from Prolabo (Merck-Eurolab, Fontenay s/bois, France). 6β-hydroxycortisol (6β-OHC), cortisol (FC), 6β-hydroxycortisone (6β-OHE) and dexamethasone were purchased from Sigma (St. Louis, Missouri, USA) and were of at least 98% purity. 6β-OHC, FC, 6β-OHE and dexamethasone were reconstituted as 1 mg/ml solutions in ethanol and stored at −20 °C. Fig. 1 shows chemical structure of the two compounds of interest; cortisol and 6β-hydroxycortisol.

2.2. Liquid–liquid extraction

The internal standard (dexamethasone) was added to 2 ml urine samples, which were vortex-mixed. Liquid–liquid extraction was then performed with an ethyl acetate/isopropanol mixture (85/15: v/v), repeated twice with 4 ml. Different extraction protocols were compared, e.g. running the extraction once 8 ml vs. twice 4 ml or using ethyl acetate alone as the extraction solvent.

After mixing for 5 min at 45 rpm, the tubes were centrifuged for 5 min at 3200 *g*.

To eliminate as much as possible other compounds extracted from urine, the organic layer was washed with basic solution (sodium hydroxide 1.0 N). Therefore, the whole organic layer was transferred into a tube containing 2 ml of NaOH 1 N. After mixing for 5 min at 45 rpm and centrifugation for 5 min at 3200 *g*, 5 ml of the organic layer were separated and then evaporated at 56 °C under a stream of nitrogen. The residue was dissolved in 200 μl of ammonium acetate buffer (10 m*M*, pH 4) and vortex-mixed for 1 min. Twenty-five microlitres of chloroform were then added to clarify the sample, and it was again centrifuged for 5 min at 3200 *g* after brief vortexing. Eighty microlitres of the aqueous layer were finally injected onto the HPLC system.

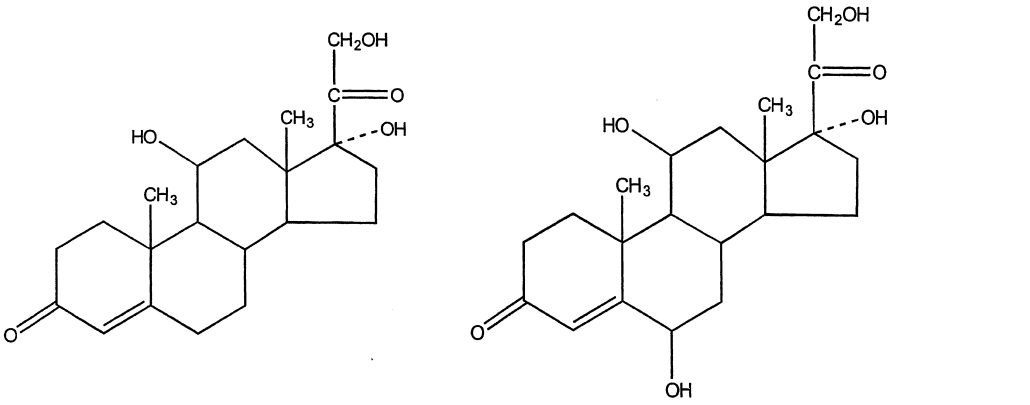

Fig. 1. Chemical structure of cortisol and its metabolite 6β-hydroxycortisol after oxidation via cytochrome P450 3A4.

2.3. HPLC apparatus and conditions

HPLC was carried out using Kontron (UVK-Lab Service, Trappes, France) 422 and 422 S coupled pumps, an autosampler 465, a C_8 reversed-phase column (Symmetry Shield RP_8, 4.6×150 mm, 5 μm; Waters, Paris, France) and the corresponding guard column (3.9×20 mm, 5 μm; Waters). Detection was performed with an UV Kontron detector 430. Detection wavelength was fixed at 244 nm. Mobile phase A consisted of an ammonium acetate buffer (10 m*M*, pH 4)/acetonitrile (95/5: v/v). Mobile phase B consisted of acetonitrile 100%. Chromatography was achieved at ambient temperature with a flow-rate of 1 ml/min by the following gradient profile: 0–50% B (linear) for 45 min.

2.4. Assessment of the purity of the peaks

In order to verify 6β-OHC and FC peaks purity, an HPLC system coupled with a diode array detector (Perkin-Elmer Serie 200, Courtabœuf, France) was used. Other conditions (i.e. column, mobile phase, and gradient) were exactly the same.

2.5. Method validation

For all the experiments relative to the method validation (spiked urines), the matrix consisted of 1/50 diluted urine. Effectively, urine could not be directly used because of endogenous compounds that could interfere with the quantification. Actually, identical results were found with pure water used as matrix.

2.5.1. Mean analytical recovery

6β-hydroxycortisol, cortisol and internal standard dexamethasone recoveries were investigated. Different concentrations were analysed. Peak areas measured from the control extraction with known added quantities of standards were compared to identical quantities of standards added to ammonium acetate buffer of mobile phase, non-extracted and directly measured. The recovery was calculated as the ratio of the control extraction to the identical non-extracted solution.

2.5.2. Linearity

6β-OHC, FC and dexamethasone were dissolved in ethanol at a concentration of 1 mg/ml and stored at −20 °C. Standards solutions were prepared by further dilution of the appropriate standard into 10 m*M* ammonium acetate buffer (pH 4). Urine standards were prepared by the addition of 50 μl of the 6β-OHC standard solution, 50 μl of the FC standard solution, 50 μl of the internal standard solution to 1900 μl human urine previously diluted 1/50 in milli-Q water to prevent interference with existing endogenous substances. Standards concentrations ranged from 10 to 5000 ng/ml and 5 to 2500 ng/ml, for 6β-OHC and FC, respectively.

Calibration graphs were obtained using the least-

squares method. Peak-area ratios of each analyte and its corresponding internal standard were used to set up the least-squares regression lines. We determined the concentration of 6β-OHC and FC in urine, using the peak-area ratios obtained from unknown samples, by interpolation from the graphs.

2.5.3. Precision

Repeated extractions ($n=5$) were performed on a single day to establish the within-day coefficient of variation (precision). The between-day coefficient of variation was determined by the same way ($n=5$). Standards were assayed in series of increasing concentration.

2.5.4. Accuracy

Repeated extractions of the same concentration ($n=5$) were performed on a single day to establish the mean accuracy. The accuracy was expressed as the ratio of the quantity of added compound to that measured.

2.5.5. Detection and quantitation limits

The limits of detection (LOD) and quantitation (LOQ) were determined for 6β-OHC and FC. For both, LOD was determined at a signal/noise=4.5. The LOQ is the lowest level of calibration.

2.6. Clinical sample collection

To evaluate the interest of our method consisting in evaluating CYP3A4 activity based on measurement of urinary 6β-OHC/FC ratio, 12 healthy Caucasian subjects collected a morning spot-urine. The subjects were non-smokers and had to take no drug for at least 15 days prior to or during the study.

Morning spot urine samples (08.00–09.00 h) were collected 1 h after the first void. Urine samples were collected for 3 different days, and a mean basal ratio and standard deviation were determined. Among them, 10 subjects were tested for urinary 6β-OHC/FC ratio the day after 600 ml grapefruit juice intake (as 200 ml 3 times a day with at least 4 h between each 200 ml intake).

3. Results

Typical chromatograms for detection of 6β-OHC and FC are displayed in Fig. 2. They were obtained from the second void morning-spot urine (between 8 and 9 am) under the recommended analytical conditions, and also a chromatogram under the same conditions of analysis on urine collected at 4 pm. Comparison shows that measuring the ratio on the second void morning-spot urine provides a chromatogram which is easy to analyse with relatively high quantities of the target species and few interferences.

Blank and spiked urine are displayed. To provide adequate calibration curves, urine could not be spiked because of endogenous species presence. For that reason, we used urine diluted 1/50 to establish the calibration curves. Urine diluted 1/50 shows no peaks that interfere with the spiked species.

3.1. Selection of the extraction solvent

Table 1 displays extraction recoveries using ethyl acetate alone as extraction solvent versus ethyl acetate/isopropanol mix (85/15: v/v). Compared to ethyl acetate alone, the combination provides good FC and dexamethasone extraction recoveries, an improved extraction recovery of 6β-OHC and a better reproducibility.

3.2. Volume of extraction solvent

Extraction in urine was performed twice with 3 or 4 ml of ethyl acetate. Double extraction with 4 ml of ethyl acetate provided a better extraction yield for the two compounds than a double with 3 ml (81% and 79% versus 67% and 73% for 6β-OHC and FC, respectively).

3.3. Choice of the internal standard

Different internal standards were tested. First, 6β-hydroxycortisone (6β-OHE) gave relatively good extraction yield and peak resolution but, on morning spot urine after grapefruit juice intake, some peaks interfered with 6β-OHE.

We then tested dexamethasone, which has a

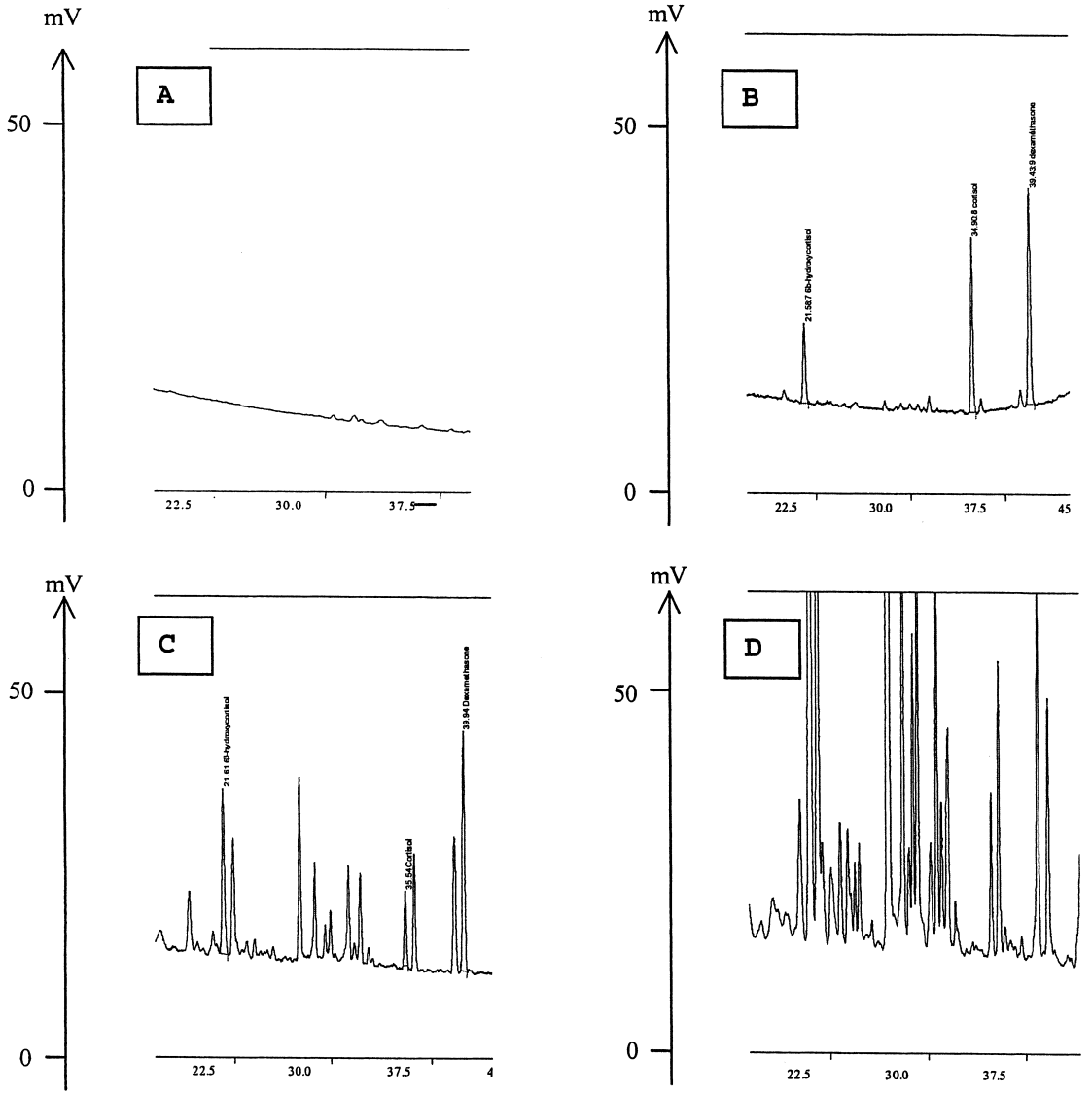

Fig. 2. Standards chromatograms. For chromatograms A and B, the matrix consisted of 1/50 diluted urine to bypass the risk of interference with endogenous compounds. A: blank. B: spiked urines with 6β-OHC 100 ng/ml, FC 100 ng/ml and dexamethasone as internal standard. These spiked urines were used to assess calibration curves. C: second-void morning spot urine (8:30 am) of a healthy volunteer. D: 4:00 pm spot urine of a healthy volunteer.

chemical structure similar to FC and so would be expected to elute close to FC, limiting the risk of grapefruit juice-induced interfering peaks. Extraction yield was quite reproducible (67%±1) and resolution was $R>1$. No peak interfered with dexamethasone, which was thus selected as internal standard.

3.4. Peak purity

6β-OHC and FC peak purity was determined from the urine of a healthy volunteer, with a diode array detector. The spectral application program TurboScan uses Perkin-Elmer's proprietary algorithm to

Table 1
Mean recovery (%) of the different substances with 2 solvents of extraction: EA (ethyl acetate) versus EA/IP (ethyl acetate/isopropanol) (85/15: v/v)

	6β-OHC		FC		Dexamethasone	
	EA	EA/IP	EA	EA/IP	EA	EA/IP
Mean recovery (%)	69	82	65	70	58	67
SD (%)	10	1	5	2	3	1
% C.V.	15	1.2	7.7	2.8	5	1.5

For each species, mean recovery was determined from 5 different concentrations (i.e. between 10 and 5000 ng/ml for 6β-OHC and 5 and 2500 ng/ml for FC), and then pooled.

calculate peak purity [24]. A Purity Index (PI) is displayed and the peak is pure if the PI value is between 1.00 and 1.50. 6β-OHC and FC peaks PI values were 1.43 and 1.12 respectively and consequently could be considered pure.

3.5. Method validation

3.5.1. Mean analytical recovery

The mean recovery was 82±1% (n=15) for 6β-OHC, 70±2% (n=15) for FC and 67±2% (n=15) for dexamethasone. Results are displayed in Table 1.

3.5.2. Linearity

Quantitation of 6β-OHC and FC was obtained from calibration curves in which the peak area ratio compound/internal standard was plotted against the compound concentration. The relationship was linear between the peak area ratios of 6β-OHC and FC over the concentration range 10 to 5000 ng/ml and 5 to 2500 ng/ml for 6β-OHC and FC, respectively. All regression coefficients r for the calibration curves were over 0.999 (n=5).

3.5.3. Precision, accuracy

The data for the validation of the within-day and between-day precision are presented in Table 2. The results show low coefficients of variation even for low urine levels of 6β-OHC and FC. Accuracy, expressed as the ratio of compound added to that measured is also displayed in Table 2. At very low levels, accuracy is very good for FC, a little less than expected for 6β-OHC, but remaining quite acceptable. Moreover, we use the ratio of 6β-OHC/FC and not the absolute concentration.

3.6. 6β-OHC/FC ratio change after white grapefruit juice intake

We investigated effect of white grapefruit juice on urinary 6β-OHC/FC ratio in conditions of inhibition after ingestion of white grapefruit juice (WGJ). Distribution of this ratio in basal conditions in 12 healthy volunteers is displayed in Table 3. Table 3 also shows the decrease of the ratio observed in 10 of these 12 healthy volunteers who drank 200 ml of WGJ 3 times over the day. The reduction range is comprised between −30% and −70%. However, one of the 10 patients had no significant modification of his urinary 6β-OHC/FC ratio.

4. Discussion

The aim of the study was to develop a simple method to rapidly evaluate CYP3A4 activity, in routine practice in every laboratory and the urinary 6β-OHC/FC ratio appeared the most suitable one.

Various methods have been described, such as high-performance liquid chromatography (HPLC) [17,18,21,23,25], gas chromatography [19], radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) [26,27]. However, immunoassays suffer from relatively poor specificity due to cross-reactivity of antibodies. HPLC methods appear to provide a more accurate selective and sensitive quantification of steroids. In the HPLC methods reported, detection is run with UV [20,21], fluorescence [25] or coupled mass-spectrometry [17–19]. However, a previous derivatization is necessary for fluorescence detection, making that technique tedious and time-consuming, and a specific apparatus is required for mass-spectrometry detection. Thus, the HPLC method coupled with UV detection appears as the simplest method. Table 4 displays the differences between three reference HPLC methods followed by UV or fluorescence detection.

Some improvements have been attempted from the first described methods. Tran et al. [14] modified

Table 2
Intra-day precision and inter-day precision

Intra-day precision					
6β-OHC					
Theoretical values (ng/ml)	10	40	200	1000	5000
Mean experimental values (ng/ml)	12.8	46.9	232	1081	5075
SD (ng/ml)	0.8	1	3	15	151
C.V. (%)	6.5	2.33	1.29	1.39	2.98
Accuracy (%)	28	17	16	8	1.5
FC					
Theoretical values (ng/ml)	5	20	100	500	2500
Mean experimental values (ng/ml)	5.3	21.4	107.5	505.7	2552
SD (ng/ml)	0.2	0.3	0.7	5.6	28.9
C.V. (%)	3.8	1.6	0.67	1.11	1.13
Accuracy (%)	6	7	7.5	1	2
Inter-day precision					
6β-OHC					
Theoretical values (ng/ml)	10	40	200	1000	5000
Mean experimental values (ng/ml)	12.5	46.8	222	1086	5004
SD (ng/ml)	0.5	0.5	8	36	168
C.V. (%)	4	1	3.6	3.3	3.3
Accuracy (%)	25	17	11	9	0.1
FC					
Theoretical values (ng/ml)	5	20	100	500	2500
Mean experimental values (ng/ml)	5	20.2	106.9	525.9	2545
SD (ng/ml)	0.4	1.4	4.6	23.3	83
C.V. (%)	8	7	4.3	4.4	3.3
Accuracy (%)	0	1	6.9	5	1.8

Table 3
Urinary 6β-OHC/FC ratio in basal conditions in 12 control subjects [before white grapefruit juice (WGJ) intake] and after 200 ml WGJ intake three times a day for 10 of these 12 volunteers

Subject Identification	Urinary 6β-OHC/FC ratio		% decrease
	Before WGJ intake Mean±SD	After 600 ml WGJ (3×200 ml)	
1	9.9±1.2	6.9	30
2	8.4±1.7	4.3	49
3	7.5±1.5	6.0	20
4	5.8±1.2	2.0	66
5	4.6±0.1	3.5	24
6	4.5±1.2	3.2	29
7	3.6±0.8	ND	
8	3.3±0.9	0.9	73
9	3.2±1.4	1.4	56
10	3.1±0.8	3.5	0
11	2.5±0.3	ND	
12	1.4±0.1	0.7	50

Before grapefruit juice intake, results are displayed as the mean ratio throughout 3 days±SD. ND=Not Determined.

Table 4
Comparison of methodology between different HPLC methods for urinary measurement of cortisol and 6β-hydroxycortisol

Preparation/extraction	Mobile phase	Column	Detection	Recovery	Ref.
Solid/liquid + 2 washing steps	gradient 30 min	30 °C	UV	70.8% [6βOHC] 90.6% [FC]	[18]
Liquid/liquid (3 extraction steps) +2 washing steps	gradient 40 min	55 °C	UV	89% [6βOHC] 90% [FC]	[20]
Derivatization+solid/liquid extraction	isocratic>45 min	2 serial columns	fluorescence	92 to 107% for [6βOHC] and [FC]	[22]

Lykkesfeldt's method [21], but, actually, the method they reported was as complicated as the previous one (solid/liquid extraction followed by washing steps) and moreover, was more expensive to run because of the use of extraction cartridges. Yamamoto et al. [22] used a modified version of Joellenbeck's method [23] that remained as long (3 extraction steps+2 washing steps) and moreover, required different conditions of analysis for the two species.

Authors who developed LC–MS methods [17–19] pointed out the problem of HPLC coupled UV detection that could generate biased results due to urine compounds interfering with peaks of interest. Certainly, LC–MS methods can provide specific detection in comparison with UV detection, but the apparatus is costly and that approach cannot be done everywhere due to the availability of the technique. Furthermore, most of the authors who developed HPLC methods worked with 24 h urine samples or urine samples providing a lot of products. In our case, to bypass this problem we focused on optimising the type of urine sample that could provide the highest quantity of species with the lowest quantities of impurities. In the literature, two approaches have been developed: 24 h urine collection and single morning spot urine sample. The first one is time consuming and may be hampered by potential errors such as inadequate urine collection. For the second, excretion of 6β-OHC and FC show important diurnal rhythms, but both are so close that no significant variations of the urinary ratio of 6β-OHC to FC have been reported within the day [14,16]. Correlation between morning spot and 24 h urinary ratios have been previously performed and provided conflicting results [15,28]. In fact, different methods of morning spot urine collections were used, mainly first or second morning voids, that consisted of very different times of urine production, approximately 6 to 8 h or 1 h, respectively [14]. Thus, certain authors assumed that morning spot urine ratio is able to assess intrapatient variability, such as induction or inhibition, but it may fail in the estimation of interpatient variability, presumably because of the small amount of endogenous substrate and the lack of sensitivity of the HPLC methods used [22].

We reached our objective collecting the second morning spot urine 1 h after the first one, between 8 and 9 am. We had the highest levels of species during the day as described by Ohno et al. [15] and, because of clear urine, we obtained very acceptable chromatograms. Moreover, to confirm that no other compound excreted in urine could interfere with 6β-OHC and FC peaks, we analysed, in the same conditions, the second morning spot urine of a healthy volunteer with HPLC with a diode array detector. The spectra of each species showed that no peak interfered with them, supposing the acceptable selectivity of our method.

Compared to other techniques of extraction, such as solid/liquid extraction (SLE) followed by washing steps, we preferred a liquid/liquid extraction (LLE). Though certain authors who developed SLE technique [14,21] have found it simpler than LLE [22,23], we find that it would be of interest to have an optimized LLE technique that is easy, can be performed with only basic solvents, and is cheaper than SPE. Our method allowed us to reduce the number of steps to 3: 2 extractions and 1 washing vs. 3 extraction steps and 2 washing steps in Joellenbeck's method [23].

Solvent extraction consisted of an ethyl acetate/isopropanol mix (85/15: v/v) that provided acceptable and reproducible recoveries for the 2 compounds: 82±1% and 70±2% for 6β-hydroxycortisol and cortisol, respectively. This last point is worthy of mention; when calculating a ratio between two species, the two percentage recoveries have to be quite reproducible and close. Actually, Lykkesfeldt's

method provided rather different recoveries, 90 and 70% for the two species.

Elution was performed with a 45 min gradient of acetonitrile 5–55% in an ammonium acetate buffer 10 m*M*, pH 4 and UV detection allowed a very large and accurate range of quantitation of the two species from 10 to 5000 ng/ml for 6β-OHC and from 5 to 2500 ng/ml for FC. Unlike the above-described methods, the analysis was run at room temperature and did not require a thermostated column.

Urinary 6β-OHC/FC ratio was measured in 12 healthy volunteers. The ratio ranged between 1.4 and 9.9 (mean=4.8±2.6) in accordance with previous studies in the literature (5.5±1.6 [18]; 7.8±3.99 [22] or 6.2±1.6 [23] all with ranges similar to our results).

Out of the 12 healthy volunteers tested for basal urinary 6β-OHC/FC ratio, 10 were then tested for urinary 6β-OHC/FC ratio after white grapefruit juice ingestion, known as a CYP3A4-suicide inhibitor. Because of this type of inhibition, WGJ effect is irreversible and still significant 24 h after its ingestion [6,29]. Our HPLC method enabled us to easily follow morning-spot urinary 6β-OHC/FC ratio during prolonged white grapefruit juice ingestion (data not shown). Actually, conditions of analysis, such as the length of the gradient, the internal standard, the composition of the extraction solvent and that of the mobile phase, were carefully set up to get rid of potentially interfering peaks generated by grapefruit juice.

Most of the studies of CYP3A4 inhibition by WGJ showed that only intestinal CYP3A4 seemed to be the subject of the inhibition. WGJ has nevertheless been commonly used with the HIV protease inhibitor saquinavir to increase the oral bioavailability of this molecule [30]. Effectively, some authors compared AUC (Area Under the concentration vs. time Curve) of felodipine [29,31], midazolam [32] or cyclosporine A [33], before and after WGJ ingestion. In all the cases, comparison was made between oral and intra-venous administration. AUC was elevated after WGJ ingestion with oral administration but not with intra-venous administration. Those authors concluded an exclusive effect of WGJ upon intestinal CYP3A4.

However, we found that the urinary 6β-OHC/FC ratio could be reduced from −30 to −70%, after three daily ingestions of 200 ml (a total of 600 ml within a day) of WGJ. The urinary ratio is likely to be more reflective of hepatic CYP3A4 activity than intestinal activity. We can explain our conflicting results with those of the literature by several arguments. First, in all these studies, the [31] quantities of WGJ ingested were always lower than the volume used in our study. The volume of WGJ ingested is significant when active substances have to reach hepatic enzymes. Second, all these studies use WGJ as a reconstituted frozen concentrate and for our study, we used fresh WGJ commercially squeezed. Even if compounds such as furanocoumarine dimmers (di-hydroxybergamottine) have been identified as potentially good candidates to explain CYP3A4 inhibition, a consensus does not exist and interactions between various compounds constituting WGJ seem to be implicated [5,6]. Third, when comparing the influence of WGJ ingestion on AUC of a drug administered orally or intra-venously, the main pharmacokinetic parameters have to be seriously considered. For example, Ducharme et al. [33] said that cyclosporine A was poorly extracted by the liver with minimal first-pass hepatic metabolism. In that case, a modification of hepatic CYP3A4 activity would have little impact on the disposition of the drug.

From now on, we plan to use fresh WGJ as a pharmacological modulator of some orally administered anticancer drugs mainly metabolised by CYP3A4. This down modulation could be of interest in patients with advanced cancer, progressive on treatment, not truly refractory to these drugs, but rather because of accelerated metabolism through CYP3A4. Limited sampling strategy and pharmacokinetics of population could help early detection of patients who have high CYP3A4 levels of activity and who could benefit from white grapefruit juice continuously added to their anticancer treatment.

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