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Nonaqueous capillary electrophoresis method for the analysis of gleevec and its main metabolite in human urine

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Abstract

The viability of nonaqueous capillary electrophoresis (NACE) was investigated for determination of gleevec and its main metabolite in human urine using a fused-silica capillary. Baseline separation of the studied solutes was obtained using a nonaqueous solution composed of 12 mM ammonium acetate and 87.6 mM acetic acid in methanol–acetonitrile (ACN) (80:20, v:v) providing analysis time shorter than 3 min. Different aspects including stability of the solutions, linearity, accuracy and precision were studied in order to validate the method in the urine matrix. Detection limits of 24 $\mu g\,L^{-1}$ for gleevec and its metabolite were obtained. A robustness test of the method was carried out using the Plackett–Burman fractional factorial model with a matrix of 15 experiments. The developed method is simple, rapid and sensitive and has been used to determine gleveec and its metabolite at clinically relevant levels in human urine. Before NACE determination, a solid-phase extraction (SPE) procedure with a C_{18} cartridge was necessary. Real determination of these analytes in two patient urines were done.

Keywords: Nonaqueous capillary electrophoresis; Gleevec; Imatinib mesylate; Ruggedness; Human urine

1. Introduction

Gleevec (imatinib mesylate, also known as STI-571) is the first of a new class of antiproliferative agents called signal transduction inhibitors, which interfere with the pathways that signal the growth of tumor cells. Gleevec is targeted to the specific biochemical abnormality found predominantly in a form of leukemia called chronic myeloid leukemia (CML). Gleevec kills the abnormal cells while having little effect on normal cell growth. Laboratory studies have shown that gleevec also inhibits an enzyme called C-Kit which is present in a relatively rare form of cancer called gastro intestinal stromal tumor (GIST).

Recently, gleevec was approved by the US food and drug administration (FDA) in record time [1] for the treatment of patients at any of the three stages of CLM.

Gleevec is chemically designated as 4-[(4-methyl-1-piperazinyl)methyl]-*N*-(4-methyl-3-{[4-(3-pyridinyl)-2-pyrimidinyl]amino}-phenyl)benzamide thanesulfonate. The main circulating active metabolite in humans is the *N*-demethylated piperazine derivative (Fig. 1). The plasma concentration for this metabolite is about 15% of the concentration for imatinib and the terminal half life is approximately 40 h at steady state. There is no change in the kinetics of this metabolite on repeated dosing and it did nor unexpectedly accumulate after long term administration of gleevec to patients [2].

Based on the recovery of compounds after an oral ^{14}C-labelled dose of imatinib, approximately 81% of the dose was eliminated within 7 days in feces (68% of dose) and urine (13% of dose). Unchanged imatinib accounted for 25% of the dose (5% urine, 20 feces), the remainder being metabolites [3].

The recommended dosage of gleevec is 400 mg/day for patients in chronic phase CML and 600 mg/day for patients in

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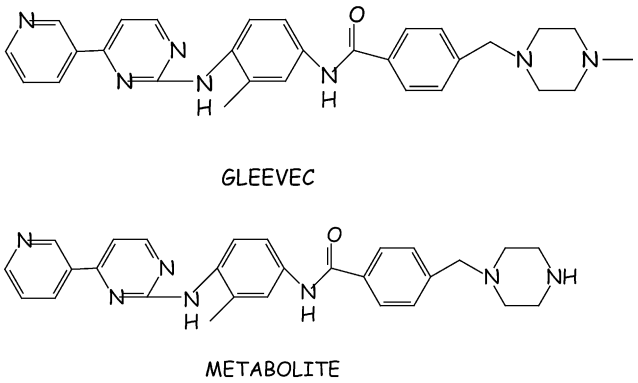

Fig. 1. Structures of the studied molecules.

accelerated phase or blast crisis. The prescribed dose should be administered orally.

Liquid chromatography–tandem mass spectrometry has been demonstrated to be an useful technique for study and determination of the antileukemia drug (gleevec) and its main metabolite in human plasma [4–6] and in monkey plasma using a semiautomated solid-phase extraction (SPE) procedure [7]. So, an isocratic reversed-phase liquid chromatography method with UV detection has been developed for the evaluation of imatinb mesylate in bulk drug [8]. A voltammetric method for total determination of gleevec and its metabolite in urine samples [9] has been developed, and the same authors have been using capillary electrophoresis using to determine gleevec and its main metabolite (*N*-demethylated piperazine derivate) in urine [10], in this work, the authors use an electrolyte consisting on 100 mM phosphoric acid adjusted to pH 2 with the addition of triethanolamine and they obtained detection limits of 0.1 mg L^{-1}. In this method, a dilution of the sample was the only step necessary before the electrophoretic analysis, but the obtained electropherogram shows a lot of urine peaks and a very bad resolution between gleevec and its metabolite in same urines. To date, NACE has not yet been used to determine gleevec, though it offers some advantages.

In nonaqueous capillary electrophoresis (NACE) an electrolyte is dissolved in either one organic solvent or a mixture of several organic solvents to carry out zone electrophoresis in fused-silica capillaries. The use of nonaqueous buffers to extend the application range of CE, has found growing interest. Compared to aqueous buffer solutions, the different physicochemical properties of organic solvents (viscosity, dielectric constant, polarity, autoprotolysis constant, electrical conductivity, etc.) induce selectivity modification in the separation process. In fact, organic solvents proved to be useful for the analysis of hydrophobic compounds, which are difficult to separate with aqueous buffers. High efficiency and resolution, shorter analysis time and the possibility to increase analyte solubility are the main reasons for the use of these organic solvents.

In this work, we have developed a simple, sensitive, rapid and robust method that allows to determine gleevec and its main metabolite in urine at clinical levels by NACE. For the first time, NACE is utilized for determining this drug, and the advantage of this method over the electrophoretic method proposed by Rodríguez et al. is the obtaining of lower detection limits, a cleaner electropherogram, without matrix interferences.

2. Experimental

2.1. Equipment

Analysis was performed with Beckman P/ACE System 5510 capillary electrophoresis equipment (Palo Alto) with diode-array detection (DAD) and controlled by a Dell Dimension P133V with P/ACE station software. The 37 cm (30 to the detector) × 75 μm i.d. fused-silica separation capillary was maintained in a cartridge with a 100 μm × 800 μm detection window.

The extraction and preconcentration process was achieved with a home-made device composed by Waters manifold Millipore Vacum sep-pack system coupled with a Gilson Minipuls 3 automatic pump (Milford, MA, USA).

2.2. Chemicals

Methanol and acetonitrile (ACN) (HPLC grade) was purchased from PANREAC (Madrid, Spain).

Gleevec and its main metabolite (*N*-demethylated piperazine derivative) were obtained from Novartis Pharma ag, Basel, Switzerland. Fluoxetine hydrocloride (FXT) was supplied by TOCRIS (Bristol, UK).

Standard solutions (200 mg L^{-1}) were prepared in methanol and stored under refrigeration at 4 °C. Working standard solution were prepared daily by dilution of the stock standard solutions with methanol.

2.3. Procedure

2.3.1. Nonaqueous capillary zone electrophoretic (CZE) separation

Uncoated capillaries were used throughout. Prior to use they were conditioned by flushing with 0.1 M NaOH for 20 min, water for 15 min and 10 min with the separation electrolyte.

At the start of each sequence of analyses, the capillary was washed for 5 min with 0.1 M NaOH, 5 min with water, and 5 min with running electrolyte. The applied potential was 20 kV, average current of 19.4 μA and the capillaries were thermostated at 22 °C. The detection was performed at 240 nm. The injections of the samples were hydrodynamically (0.5 psi; 1 psi = 6894.76 Pa) for 6 s.

The running electrolyte was based on methanol–acetonitrile (80:20; v/v), containing 12 mM ammonium acetate and 87.6 mM acetic acid. The current was in range of 20–25 μA. Electrolyte solutions were prepared freshly

and not used after long storage periods. Between measurements, the capillary was conditioned for 1 min with running buffer because contamination from an unknown component of the urine samples was observed between consecutives injections.

Duplicate injections of the solution were performed and relative peak areas (analyte area/fluoxetine area) were used for the quantification.

As the electrolysis of the electrolyte solution can occur and subsequently change the electrosmotic flow (EOF), the separation electrolyte was replaced every six injections. When the capillary was not in use, it was washed with 0.1 M NaOH, water and dry-stored.

2.3.2. Extraction and preconcentration procedure

Due to the presence of a large quantity of various interfering compounds it was necessary to extract the compounds of interest in order to obtain a cleaner electropherogram. The extraction of gleevec and its main metabolite from the urine samples was performed in a reversed-phase C_{18} cartridge (Waters Sep-Pak Plus, Milford, MA, USA). The cartridge was conditioned prior to use with 5 mL of methanol followed by 5 mL of 10 mM phosphate buffer solution (pH 7.0).

Variables such as organic solvent, washing stages using different solvents, organic-water ratio for elution of the analytes free from interferences, and final volume of the extract, were studied.

Sample passage, different volumes (between 2 and 10 mL) of urine were slowly loaded into the conditioned cartridge. Once the retention step had been completed, the cartridge was washed with 8 mL of 10 mM phosphate buffer (pH 7.0) and 2 mL of a 30% methanol–water (30:70) solution in order to obtain a cleaner electropherogram without any matrix interference. Finally, elution of the retained components was achieved with 2 mL of methanol. The maximal capacity of the cartridge was investigated and established in 10 mL of urine, therefore, it was possible to preconcentrate five times.

Quantification was performed by evaluating the normalized area of each studied compounds versus the internal standard area (fluoxetine).

2.3.3. Treatment of the urine samples

Fresh human urine samples were obtained from different volunteers who had or had not taken gleevec.

Fresh urine samples were submitted directly to solid-phase extraction after a preliminary centrifugation step (5000 rpm, 15 min, 20 °C).

2.4. Stability of the solutions

Although this test is often considered as part of the ruggedness of the procedure, it should be carried out at the beginning of the procedure because it determines the validity of the data of the others test.

The stability of the standard and test solutions of gleevec, its metabolite and fluoxetine (I.S.), was determined by comparing the response factors (concentration/average peak areas) of triplicate solutions stored at 4 °C, in darkness, with those of freshly prepared triplicate solutions. The difference between the concentrations of freshly prepared solutions and those aged from 15 days was <1.5%, and the absorption spectra of the solutions were found to be unchanged during this period. In this way, stock standard solutions were checked and found to be stable for at least 1 month.

The stability of spiked urine extract containing gleevec, its metabolite and fluoxetine was evaluated by comparing the relative peak areas obtained at different time intervals with those of a freshly prepared extract. It was found that the extract was stable for at least 4 h.

3. Results and discussion

3.1. Optimisation of the test electrophoretic procedure

3.1.1. Effect of the nature of electrolyte

The pH of the electrolyte solution plays an important role in CE of weak acids and bases. Most organic solvents exhibit greater solvation strength than water, so that CE can be extended to the analysis of hydrophobic substances [11]. The most common buffering system in organic solvents consist of acids and their ammonium salts, of which acetic acid and ammonium acetate certainly have been most frequently used [12].

A mixture of NH_4OAc and HOAc was also used in present study.

The optimisation of the procedure was carried out with an urine extract that contained 5 mg L^{-1} of gleevec, its metabolite and fluoxetine (internal standard). The effect of the concentration of NH_4OAc (5–15 mM) on the migration time of the investigated compounds was studied, maintaining 262.8 mM of HOAc constant in all the prepared electrolytes. As expected, when the concentration of NH_4OAc increases the migration times of gleevec and its metabolite also increase.

A concentration of 12 mM NH_4OAc buffer was selected as optimal since this value maintains good peak shape, relatively low current (<20 μA) and good resolutions between peaks. Fig. 2 shows that as the concentration of NH_4OAc is increased, resolution between peaks also increased.

In the same way, the influence of increasing amounts of acetic acid (0–262.8 mM) over resolution was studied maintaining 12 mM of NH_4OAc constant. The acidity of the electrophoretic medium is also a governing factor in separation of the ionisable analytes because it determines the extent of ionisation of each individual analyte in NACE. Low concentrations of acetic acid in the buffer increase resolution between peaks, but also increase migration times of the studied drugs. A value of 87.6 mM was chosen as optimum be-

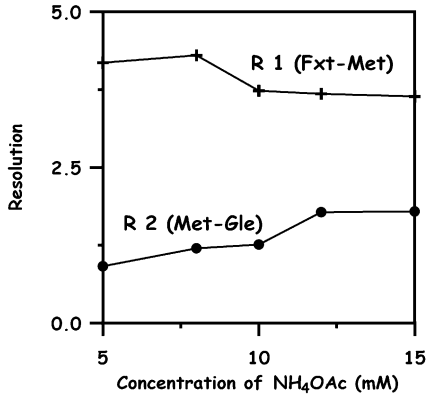

Fig. 2. Effect of concentration of NH_4OAc on resolution between peaks. R1 is resolution between fluoxetine and the metabolite. R2 is resolution between the metabolite and gleevec.

cause it provides a good resolution with a short analysis time.

3.1.2. Effect of ACN–MeOH mixtures percentages

ACN–MeOH mixtures are widely used in NACE. The selectivity of the separation systems has reported to change significantly with the ratio of ACN and MeOH [13].

Several ACN–MeOH mixtures containing 12 mM NH_4OAc and 87.6 mM HOAc were tested for the separation of the studied compounds. As it can be seen in Fig. 3, when the % of ACN increases, migration times of gleevec and its metabolite decrease and decrease too resolution between peaks. A value of 20% of ACN was chosen as a compromise solution.

3.1.3. Selection of voltage, temperature and injection time

The effect of the voltage applied from 10 to 25 kV was investigated. A voltage of 20 kV yielded the optimal solution of run time, low generated electric current and resolution between peaks.

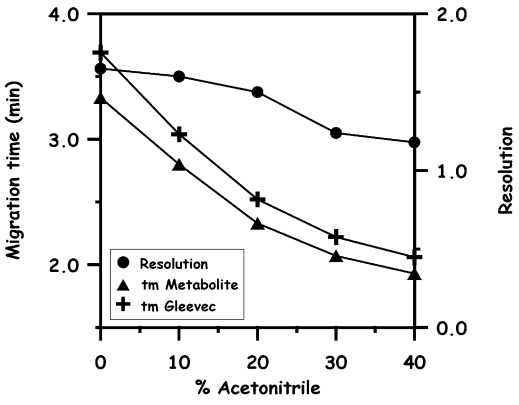

Fig. 3. Effect of %ACN on gleevec and metabolite migration times, and resolution between gleevec and its metabolite.

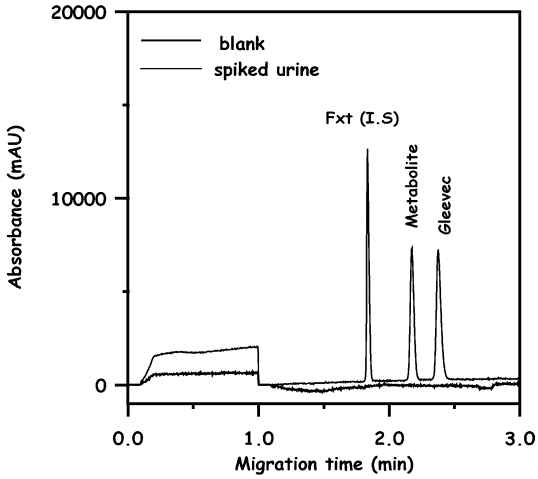

Fig. 4. Electropherograms corresponding to: a blank of urine and to a spiked urine. Operating conditions: nonaqueous system of 80:20 methanol–acetonitrile containing 12 mM ammonium acetate and 87.6 mM acetic acid, hydrodynamic injection 6 s, 20 kV and 22 °C.

The effect of temperature on the separation was investigated in the range 18–25 °C. The selected temperature was 22 °C because it provided the best resolution and the generated current was lower than 20 μA.

The injection time was varied between 2 and 10 s (injection pressure 0.5 psi). As expected, when the injection time increased the peak area of all compounds also increased, but for injection times higher than 6 s a loss of resolution between peaks was observed. For this reason, 6 s of injection time was chosen as optimal value.

Fig. 4 shows the electropherogram corresponding to the extracts from 6 mL of human urine spiked with 1 mg L^{-1} of each of the studied compounds. From this electropherogram, it can be seen that the electrophoretic procedure selected is suitable for the separation of the studied drugs.

3.2. Validacion of the NACE procedure

3.2.1. Linearity

The linearity of the response was examined by the injection of urine samples spiked with seven different concentrations ($n = 7$) after SPE treatment. The linearity was tested over the range from 0.5 to 6.0 mg L^{-1} for each substance in the urine. In all the cases 3 mg L^{-1} of fluoxetine was added as internal standard. This process was repeated on three different days. On the first day the sample was an urine of a young and healthy woman (calibration 1), on the second day the sample was a mixture of four different persons' (three young and healthy women and one old man) (calibration 2) urine and on the third day the same urine mixture after a frozen process (calibration 3) was analysed. The results were given in terms of relative peaks areas and the obtained regression lines pass through the origin in all the cases.

An analysis of variance (ANOVA) test was performed to compare the different regression lines obtained, to determine

Table 1
Linearity ($n = 7$)

	Global equation[a]	Coefficient of correlation	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)
Metabolite	$y = (-0.078 \pm 0.131) + (0.943 \pm 0.028)x$	0.997	24	80
Gleevec	$y = (0.057 \pm 0.063) + (0.823 \pm 0.036)x$	0.997	24	80

LODs and LOQs for the two study drugs.
[a] Concentration (x: $mg\,L^{-1}$) vs. relative peak area (y).

whether the data could be combined to enable estimation of appropriate quantities by use of a comprehensive regression line. F_{exp} compares the deviation between set lines with the deviations within each set from the set lines. In all the cases the experimental value of F is lower than the theoretical value of F for different urines. For this reason, we can propose a global calibration graphs with representative slopes (Table 1).

As consequence of the previous studies, determination of all the studied drugs can be performed by direct measurement from the calibration graph.

3.2.2. Recovery

In order to test the accuracy of the proposed method, several aliquots of gleevec, its metabolite and fluoxetine standard solutions were added into human urine samples. These samples were analysed using the extraction, preconcentration and electrophoretic procedures described in this work. The concentration found in the test solutions were calculated by reference to the duplicate bracketing standard solutions and the recoveries obtained (upon relative peak areas measurements with regard to the internal standard) for these test solutions are shown in Table 2. As it can be seen, recoveries (mean of three values) very close to 100% were obtained in all cases.

Recoveries of the procedure over the tested range can also be assessed from the graph of the found concentration (Y) versus the added concentration (X). The graph should have a slope of unity and should pass through the origin. The equations obtained were:

$$\text{metabolite}: \quad y = (0.030 \pm 0.065) + (0.984 \pm 0.012)x; \quad r^2 = 0.999$$

$$\text{gleevec}: \quad y = (0.004 \pm 0.139) + (0.980 \pm 0.025)x; \quad r^2 = 0.999$$

Table 2
Recovery of human urine samples[a]

Sample	Metabolite		Gleevec	
	Added ($mg\,L^{-1}$)	Recovery (%)	Added ($mg\,L^{-1}$)	Recovery (%)
1	0.5	101.9 ± 1.2	0.5	98.8 ± 1.2
2	1.0	102.5 ± 2.0	1.0	96.7 ± 1.1
3	3.0	97.3 ± 1.9	3.0	100.1 ± 1.3
4	4.0	100.1 ± 1.9	4.0	95.1 ± 1.5
5	5.0	99.9 ± 1.7	5.0	100.3 ± 1.2
6	8.0	98.7 ± 1.6	8.0	97.3 ± 2.0
7	10.0	98.5 ± 1.9	10.0	98.3 ± 1.2

[a] Mean value ± S.D ($n = 3$).

3.2.3. Repeatability and reproducibility

Two different samples containing 3 $mg\,L^{-1}$ of gleevec and its metabolite, were prepared and analysed on two different days using the optimised method. Repeatability was studied by performing series of nine separations of one of these samples. The reproducibility was studied by performing nine separations of other sample 24 h later than analysis of the first, under the same conditions but different operator and different CE equipment. The results showed that the repeatability (using relative peak areas) for two compounds on each days is satisfactory (Table 3).

To determine the precision in the recoveries of gleevec and its metabolite, a mixture of three different urine samples were spiked with three different concentration of each drug (from 1 to 3 $mg\,L^{-1}$). Excellent recoveries were obtained (between $96.5 \pm 3.5\%$ and $101.8 \pm 4.6\%$) for determination of each substance in the mixture of urine samples. Similar recoveries of gleevec and its metabolite were obtained for the same mixture of urine samples submitted to a frozen process. So, there are not significant differences between drugs recoveries from fresh or frozen urine samples.

3.2.4. Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection and quantification were calculated by measuring in six urine blanks. LODs were estimated at signal-to-noise ratio (S/N) of 3 and the LOQ was defined at S/N 10.

The LODs and LOQs obtained, taking into account a concentration factor of 5 (from 10 mL of urine it was obtained a methanolic extract of 2 mL) for all the studied compounds (from the extraction-preconcentration process), are summarised in Table 1. The LOQs were subsequently experimentally evaluated by the analysis of two different samples prepared by adding concentrations corresponding to the LOQ for each studied drug to urine samples and subjecting these samples to the analytical procedure. The

Table 3
Repeatability and reproducibility

	Metabolite		Gleevec	
	Day 1	Day 2	Day 1	Day 2
R.P.A.[a] (mean)	2.13	2.21	2.53	2.61
S.D.	0.05	0.04	0.05	0.06
R.S.D. (%)	2.12	1.82	1.82	2.30
F_{exp}	1.56		1.44	
F_{theor}	3.44		3.44	

[a] R.P.A. is relative peak area (drug area/fluoxetine area) ($n = 9$).

obtained relative errors were less than 10% in all the cases.

3.2.5. Ruggedness/robustness

Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results. A robustness test is an experimental set-up to evaluate the robustness of a method. The term ruggedness is frequently used as a synonym [14–17]. To examine potential sources of variability, a number of factors are selected from the operating procedure and examined in an interval that slightly exceeds the variations which can be expected when a method is transferred from one instrument to another or from one laboratory to another. Another aim of a ruggedness/robustness test may be to predict reproducibility or intermediate precision estimates [18]. Fractional factorial designs developed by Plackett and Burman [19] were used, based on balanced incomplete blocks according to procedures described by Youden and Steiner [14].

The choice of variables and the levels at which to test them is very important if the ruggedness test is to be of value. Variables must be those which are likely to be significant in practice, and the levels must reflect the variation which are usually observed. The variables and levels selected in our study are:

(a) voltage (18 $_{(-1)}$, 20 $_{(0)}$, 22 $_{(+1)}$) (kV);
(b) injection time (5 $_{(-1)}$, 6 $_{(0)}$, 7 $_{(+1)}$) (s);
(c) concentration of NH_4OAc (10 $_{(-1)}$, 12 $_{(0)}$, 14 $_{(+1)}$) (mM);
(d) concentration of acetic acid (43.8 $_{(-1)}$, 87.6 $_{(0)}$, 131.4 $_{(+1)}$) (mM);
(e) % ACN (15 $_{(-1)}$, 20 $_{(0)}$, 25 $_{(+1)}$);
(f) temperature of the separation (20 $_{(-1)}$, 22 $_{(0)}$, 24 $_{(+1)}$) (°C);
(g) detection wavelength (238 $_{(-1)}$, 240 $_{(0)}$, 242 $_{(+1)}$) (nm).

The three values used for each variable are shown in parentheses, where level (−1) is the minimum value studied, level (0) is the optimal value for the method, and level (+1) is the maximum value tested in this experiment.

The mean value of each variable is the average difference between observation made at the extreme levels and those made at the optimal level. Mean effects and standard errors (DA, DB, DC, . . .) were calculated using the procedures described by Youden and Steiner.

The ruggedness was determined from triplicate injections of a methanolic solution that contained 3.0 mg L^{-1} of gleevec and its metabolite in presence of fluoxetine (internal standard). Results of the effect of each factor's levels over efficacy, resolution and relative peak areas were calculated (Table 4).

Taking into account the deviations calculated for the different checked results when the selected operating factors were tested upon the experimental design of Placket–Burman and the statistical treatment of Youden–Steiner, this analytical method for measuring of gleevec and its metabolite has proved to be rugged to all the variations tested in this work. The validity of the Plackett–Burman design is confirmed for the purpose of ruggedness testing and ruggedness obtained in all cases (using the statistical method of Youden and Steiner), allows to use this method by different laboratories, analysts or instrument without any appreciable error.

3.3. Applications

To demonstrate the applicability of the extraction, preconcentration and NACE procedure developed, several urine samples of different voluntaries (some of them were patients undergoing medical treatment) were analysed.

At the first time, urine of three different voluntaries (submitted to different treatment) were spiked with three different concentrations of gleevec and its metabolite

Table 4
Deviations for each result obtained upon the statistical treatment of Youden and Steiner

	Efficacy		Relative peak area		Resolution (metabolite–gleevec)
	Metabolite	Gleevec	Metabolite	Gleevec	
$DA_{(+1)}$	2079	2175	−0.072	0.239	0.659
$DB_{(+1)}$	2014	1329	−0.099	0.247	−0.128
$DC_{(+1)}$	1543	−181	−0.070	0.092	−0.352
$DD_{(+1)}$	1255	746	0.232	0.598	0.354
$DE_{(+1)}$	919	569	0.131	0.526	0.703
$DF_{(+1)}$	1592	650	−0.034	0.170	0.504
$DG_{(+1)}$	85	−858	−0.769	−0.618	−0.652
$DA_{(-1)}$	−378	127	0.072	0.438	−0.224
$DB_{(-1)}$	−826	−2024	0.029	0.294	−0.852
$DC_{(-1)}$	1745	−288	0.016	0.472	0.443
$DD_{(-1)}$	1837	1046	−0.061	0.093	−0.489
$DE_{(-1)}$	−568	1000	−0.066	0.032	−0.647
$DF_{(-1)}$	2258	1503	0.004	0.415	0.479
$DG_{(-1)}$	−201	520	0.684	1.094	−0.099
S	2850.1	2228.9	0.577	0.934	1.034
$\sqrt{2}S$	4030.6	3152.2	0.816	1.321	1.462

Table 5
Analysis of human urine samples

	Concentration found (mg L^{-1})	
	Metabolite	Gleevec
Patient A (600 mg/day)	12.2 ± 0.2	29.9 ± 0.8
Patient B (100 mg/day)	3.1 ± 0.1	6.8 ± 0.2

(1.0–5.0 mg L^{-1}) and a constant fluoxetine concentration (3.0 mg L^{-1}) in order to evaluate possible interferences in the gleevec and metabolite determination. The patient 1 was taken oral contraceptive Mycrogynon (ethinylestradiol and levonorgestrel), the patient 2 was taken paracetamol 650 mg/8 h and the patient 3 was taken ibuprofen 600 mg/8 h. Good recoveries of gleevec (96.3 ± 2.6%) and its metabolite (94.3 ± 3.4%) were obtained, and the electropherograms shown the no interference from these drugs.

Then, urine of two different voluntaries undergoing medical treatment with gleevec were also analysed (frozen samples). The first voluntary (patient A) was a man receiving 100 mg/day of the drug and the second volunteer was a woman (patient B) receiving 600 mg/day of gleevec orally, in this case, it was necessary to dilute the urine with water in the ratio (urine:water) 1:5. Concentrations found using this procedure are shown in Table 5. These results were in agreement with results obtained by the capillary zone electrophoretic method proposed by Rodríguez et al. [10].

Fig. 5 shows the electropherogram corresponding to the urine of patient B, after dilution with water to have a concentration of the drugs in the range examined. As it can be seen, no interference from the matrix was observed.

3.3.1. Specificity

As in any separation technique, co-elution of peaks is possible in capillary electrophoresis; and therefore it was interesting and useful investigate the homogeneity or purity of the obtained peak.

In this work the techniques used to validate the peak purity of the studied compounds present in urine samples were [20]:

(i) absorbance at two wavelengths;
(ii) normalization and comparison of spectra from different peak sections.

Both techniques showed that the purity of the peaks corresponding to the compounds studied in urine present a high level of purity.

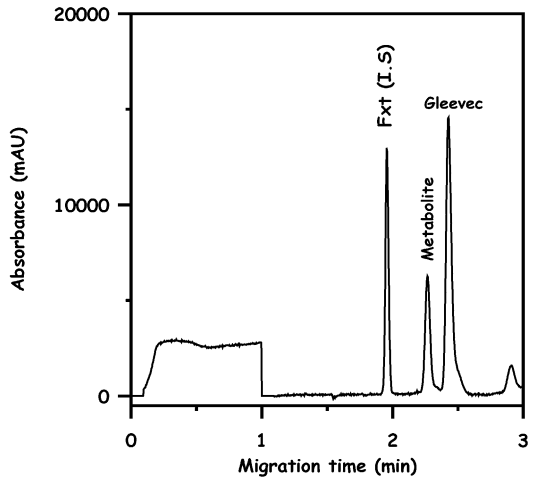

Fig. 5. Electropherogram of a urine sample from a woman on gleevec treatment, 600 mg/day. Operating conditions: nonaqueous system of methanol–acetonitrile (80:20) containing 12 mM ammonium acetate and 87.6 mM acetic acid, hydrodynamic injection 6 s, 20 kV and 22 °C.

4. Conclusion

In this work a rapid, easy, robust and sensitive method for the determination of gleevec and its main metabolite in urine by NACE is described. The electrophoretic (NACE) method has been validated for the analysis of the two compounds in human urine without any matrix interference. It has been shown that the experimental results with respect to linearity, accuracy, specificity, sensitivity, precision and ruggedness of the test validation demonstrate the reliability of the electrophoretic procedure for its intended application: quantification of the compounds under study at clinically relevant concentrations.

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