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New spectrophotometric procedure for determining cefotaxime based on derivatization with 1,2-naphthoquinone-4-sulphonate into solid-phase extraction cartridges — application to pharmaceutical and urine samples

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Abstract

Cefotaxime was derivatised with 1,2-naphthoquinone-4-sulphonate (NQS), extracted into solid-phase cartridges (C_{18}) and detected using a UV–visible detection system. Optimum conditions for this new procedure were: hydrogencarbonate–carbonate buffer, pH 10.5, 5-min reaction time at 25°C and an NQS concentration of $7.1 \cdot 10^{-3}$ mol l^{-1}. The accuracy and the precision of the liquid–solid procedure were tested. The procedure was used to measure cefotaxime in pharmaceutical and urine samples. The results obtained were contrasted with those reported for a HPLC method for urine samples. The generalized H-point standard additions method was used to measure cefotaxime in urine samples.

Keywords: Solid-phase extraction; UV-visible detection; Generalized H-point standard additions method; Cefotaxime

1. Introduction

Cefotaxime, a third generation cephalosporin, has a broad antibacterial spectrum and is resistant to β-lactamases. Several methods for cefotaxime determination in pharmaceuticals and biological fluids have been reported. High-performance liquid chromatography (HPLC) [1–7], capillary electrophoresis [2,3], thin-layer chromatography [8], differential pulse polarography and adsorptive stripping voltammetry [9] and spectrophotometric [10–14] techniques have all been used. HPLC is the most common technique for determining cephalosporins. The compendia [15–17] recommend the volumetric, spectrophotometric or HPLC methods for pharmaceutical samples. However, to our knowledge, a spectrophotometric method for determining cefotaxime in urine samples has not been reported. Some authors indicate that the UV-spectrophotometric method suffers from absorbance interferences and have proposed several colored reactions. The most frequently used reagents for cefotaxime are ammonium molybdate in acid media and iron(III), which yields coloured complexes with hydroxamic acids obtained from the reaction between the carboxylic group of the cephalosporin and some amines.

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Derivatization has long been accepted as an effective modification technique that can improve the overall specificity and sensitivity of trace analysis. The usual methodology involves a solution-based derivatization procedure. Over the past decade, immobilized solid- phase reagents on a polymeric solid support have become increasingly popular for easy conversion of analytes into more detectable species [18]. We have demonstrated the possibility of integrating sample clean-up and derivatization in the same process by using C_{18} materials such as those commonly employed for analyte purification and preconcentration. In this way, analytes can be purified, preconcentrated and derivatized with minimum sample handling. This methodology is an alternative to the use of polymeric reagents avoiding the synthesis of these reagents that is often difficult and their necessary regeneration for obtaining high yield, fewer side products and reproducible results.

Our approach has been applied to the on-line and/or off-line HPLC analysis of several amines such as amphetamines, with the derivatizing reagents 1,2-naphthoquinone-4-sulphonate [19,20], OPA [20], 9-fluorenylmethylchloroformate [20–22] and 3,5-dinitrobenzoyl chloride [23]. We have shown that this methodology can be applied for the spectrophotometric determination of amines in pharmaceutical and urine samples [24,25].

In the present report we extend our methodology to cefotaxime. A very simple continuous liquid–solid procedure for the derivatization of cefotaxime on solid-phase cartridges with NQS is proposed. It can simultaneously serve to carry out the sample clean-up and derivatization steps. The specificity of the method is achieved by using generalized H-point standard additions method (GHPSAM) [26]. The GHPSAM isolates the analyte signal from the measured analytical signal. The results obtained were contrasted with those provided by HPLC [7] for urine samples.

2. Experimental

2.1. Apparatus

All spectrophotometric measurements were done on a Hewlett-Packard (Avondale, PA, USA) HP 8452. A diode-array spectrophotometer furnished with quartz cuvettes with a 1-cm pathlength.

A Hewlett-Packard 1014 A liquid chromatograph, equipped with a diode array detector linked to a data system (Hewlett-Packard HPLC CHEMSTATION, Palo Alto, CA, USA) was used for data acquisition and storage. The system was coupled to a quaternary pump (Hewlett-Packard, 1050 Series) and an automatic sample injector (Hewlett-Packard, 1050 Series). The column was a Hypersil ODS-C_{18}, 5 mm (125×4 mm I.D.) (Hewlett-Packard, Germany). The detector was set to collect a spectrum every 640 ms (over the range 220–600 nm) and all the assays were carried out at ambient temperature.

2.2. Reagents

Solutions were prepared with distilled water and all the reagents used were of analytical grade unless otherwise stated. The 1,2-naphthoquinone-4-sulphonate stock solution was prepared by dissolving the sodium salt (Sigma, St. Louis, MO, USA) in distilled water. This solution was prepared fresh for each experiment and was stored in the dark at room temperature. A stock solution of sodium cefotaxime of pharmaceutical grade (Primafen injection, labelled to contain 1 g of sodium cefotaxime, Hoechst Farma, S. Feliu de Llobregat, Barcelona, Spain) was prepared by dissolving 0.1000 g of the solid in 100 ml of distilled water or urine. Working solutions were prepared by dilution as required. The USP iodometric method provided cefotaxime a content of 107±10% for three replicates. Acetonitrile was of HPLC grade from Scharlau (Barcelona, Spain). C_{18} (200 mg ml^{-1}) Bond Elut columns were obtained from Varian (Harbor, USA).

The NaH_2PO_4 solution was prepared by dissolving 3.5 g of sodiumdihydrogen phosphate (Probus) in 500 ml of distilled water. The pH was adjusted to 3 by adding a minimum amount of 50% H_3PO_4.

2.3. Columns and mobile-phases

The mobile-phase consisted of acetonitrile–($5 \cdot 10^{-2}$ mol l^{-1} H_3PO_4–NaH_2PO_4, pH 3), with an acetonitrile content that increased from 10% at zero time to 20% at 2 min, 20% at 6 min and 50% at 8 min. The solution was prepared daily, filtered

through a nylon membrane, 0.45 μm (Teknokroma, Barcelona, Spain) and degassed with helium before use. The flow-rate was 0.75 ml/min and 10 μl of each sample were injected.

2.4. Derivatization into the solid-phase extraction columns

C_{18} extraction columns were previously conditioned by drawing through 1 ml of methanol, followed by 1 ml of $5 \cdot 10^{-2}$ mol l^{-1} H_3PO_4–NaH_2PO_4, pH 3. Then 1 ml of H_3PO_4–H_2O (1:8, v/v) and 1 ml of sample solution containing different cefotaxime concentrations (40–179.5 mg l^{-1} or $8.4 \cdot 10^{-5}$–$37.6 \cdot 10^{-5}$ mol l^{-1}) were transferred to the column. When the cephalosporin was retained in the column, 0.25 ml of NQS reagent (1.5%, w/v) and 1 ml of hydrogencarbonate–carbonate solution 8% (w/v) at pH 10.5, previously mixed, were added. After 5 min at a temperature of 25°C, the columns were washed with 5 ml of distilled water. The reaction products (cefotaxime-NQS) were eluted from the columns with 2 ml of acetonitrile–water (1:1, v/v). The absorbance between 190 and 820 nm was registered. Absorbance was measured against acetonitrile–water (1:1, v/v) at 25°C. Three or more replicates were processed in all cases. The analytical signal was measured at 514 nm.

2.5. Standard addition methods

For the standard addition method (MOSA) [28], aliquots of urine samples (25 ml) with 40 mg l^{-1} of cefotaxime were spiked at different cefotaxime concentration levels (0–179.5 mg l^{-1}). Then 1 ml of these samples was processed according to the procedure described for standard solutions.

3. Results and discussion

3.1. Optimization of the working conditions

The retention of cefotaxime on solid-phase extraction (SPE) columns was optimized before its derivatization with NQS. C_{18} extraction columns were conditioned by drawing through methanol followed by solutions of H_2O, H_3PO_4, $NaHCO_3$–Na_2CO_3 buffer (pH 10.6) and aqueous solutions of sodium terbutilate. The best results were obtained with H_3PO_4–H_2O (1:8, v/v). In addition, solutions of cefotaxime in H_2O, H_3PO_4 and NaAcO–HAcO (pH 4.8) were passed through the column. The best retention (74±3%, $n=10$) was obtained using 1 ml of H_3PO_4–H_2O (1:8, v/v) and 1 ml of cefotaxime solution. These results are in agreement with those obtained previously [7].

Once the retention conditions had been established, the influence of the pH in the 5–10.5 range was studied. Higher pH values were not used in order to prolong the life of the C_{18} packing cartridge. A pH of 10.5 (hydrogencarbonate–carbonate solution, 8%, w/v) was selected in order to increase the reaction rate and carry out the derivatization at room temperature (25°C). This value was similar to that used for the derivatization of amphetamine and methamphetamine with NQS [24]. The signal corresponding to the NQS-cefotaxime was approximately constant after 5 min (Fig. 1).

The effect of the NQS concentration was evaluated in the $1.2 \cdot 10^{-3}$–$1.2 \cdot 10^{-2}$ mol l^{-1} range, with cefotaxime concentrations of $16.7 \cdot 10^{-5}$ mol l^{-1} (79.7 mg l^{-1}). Fig. 2 shows the absorbance as a function of the NQS:cefotaxime concentration ratio. The analytical signal increased linearly up to an NQS concentration of $4.7 \cdot 10^{-3}$ mol l^{-1} and was practically constant between $4.7 \cdot 10^{-3}$ and $9.5 \cdot 10^{-3}$ mol l^{-1}. The signal of the blank reagent increased weakly with the concentration. In order to maintain low blank interference, the reagent concentration chosen was $7.1 \cdot 10^{-3}$ mol l^{-1}. Different water volumes were passed through the column to eliminate the excess reagent and a water volume of 5 ml was selected as optimum.

Acetonitrile was selected as the elution solvent because it provided good sensitivity with a low analytical signal for the NQS reagent. Although 1 ml of solvent was enough to elute all the reaction product formed, 2 ml acetonitrile–water (1:1, v/v) was used to provide enough volume to measure the analytical signal. In this solvent the derivatives remain stable for at least 1 week stored at 4°C.

Using the parameters described above, the regression equation, calculated from the calibration graph correlating the absorbance at 514 nm versus cefotax-

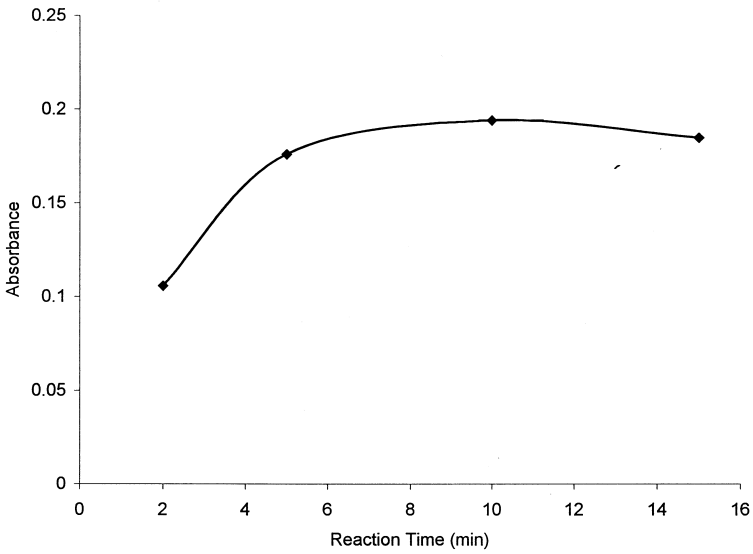

Fig. 1. Absorbance at 514 nm corresponding to the reaction product, cefotaxime-NQS, recorded against an acetonitrile–water (1:1, v/v) blank at different reaction times. Conditions: NQS $2.4\cdot10^{-3}$ mol l^{-1}; cefotaxime $5.02\cdot10^{-5}$ mol l^{-1}; pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v).

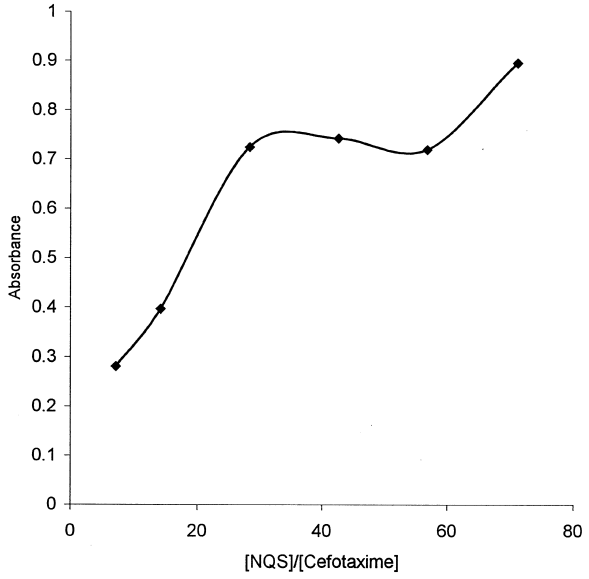

Fig. 2. Analytical signal of NQS-cefotaxime derivative versus NQS:cefotaxime molar concentration ratio. The absorbance was recorded against an acetonitrile–water (1:1, v/v) blank at 514 nm. Conditions: Cefotaxime $16.7\cdot10^{-5}$ mol l^{-1}; pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v).

ime concentration (mol l^{-1}) was: $A=(a\pm s_{a)}+(b\pm s_b)C=(0.30\pm0.04)+(5200\pm400)C$, $s_{yx}=0.06$, where s_a and s_b were the standard deviations of the intercept and the slope, respectively. The dynamic range of concentration was $8.4\cdot10^{-5}$–$37.6\cdot10^{-5}$ mol l^{-1}. The precision of the method was 6% (coefficient of variation). This value was obtained from solutions with different concentrations of the drug measured the same day. This precision was similar to those obtained by the UV–visible [14] and HPLC [7] methods. The impurity 7- aminocephalosporanic acid (7-ACA), which can be present in the trade product did not react with NQS. The 7-ACA is an interferent species in the determination of cefotaxime using the UV method because it shows an absorption band at 262 nm due to its β-lactam ring.

3.2. Urine samples

Five different urine samples were employed. Three of them were from a healthy adult volunteer and the others from an adolescent and a child. Each urine was spiked with different analyte concentrations.

The washing step after derivatization was studied. Different fractions of 2 ml (from 2 to 40 ml) of the

mixtures MeCN–water (1:39, v/v) were collected. From this study it was shown that MeCN cannot be included in the washing mixture because the recovery of the analyte is low. Therefore, 5 ml of water was selected as the washing solution. The absorbance value obtained by adding the analytical signals of urine and cefotaxime standard was similar to the absorbance value of the urine spiked with the standard (Fig. 3). Cefotaxime recovery was similar in the standard and the urine.

When MOSA was applied to the five fortified urine samples, the slopes$\pm s_b$ obtained were: $b_1 \pm s_b = 6100 \pm 300$; $b_2 \pm s_b = 6300 \pm 600$; $b_3 \pm s_b = 5800 \pm 300$; $b_4 \pm s_b = 5200 \pm 400$ and $b_5 \pm s_b = 5400 \pm 200$. These values were similar to those obtained for standard samples in the calibration graph with standards ($b = 5300 \pm 300$, $n = 3$). This indicated the absence of matrix effects. The dynamic range of concentration was $8.4 \cdot 10^{-5}$–$37.6 \cdot 10^{-5}$ mol l^{-1}. The quantification limit was $7.02 \cdot 10^{-5}$ mol l^{-1} calculated as ($10\, S_B/b$) [29], where $S_B = 0.07$ was the standard deviation of the NQS urine blank ($n = 3$, the samples processed were the three of the adult volunteer) and $b = 5700 \pm 400$ was the average slope value, $n = 5$. The limit of detection was $2.1 \cdot 10^{-5}$ mol l^{-1} calculated as ($3S_B/b$) [30].

The blanks for the five urine samples are given in Fig. 4. The NQS urine blanks are different from the NQS blank. This situation was similar to that reported for the amphetamine-NQS procedures [27]. In order to correct this bias error, we proposed in [26] the GHPSAM. The GHPSAM can be used instead of the Youden method [31]. Because the matrix effect is absent the GHPSAM can work with standard solutions instead standard additions solutions. This method is described in the appendix. Fig. 5 is an example of $A''_{s,1}/\epsilon''_{x,1}$ versus λ_j plots, which were used to identify the interval at which this quotient was constant and the interferent was therefore linear. This corresponds to a constant value for C^o. The selected intervals for the twenty-eight spiked urine samples appear in Table 1. These values are not definitive, but they enabled us to choose the linear interval in which the spectral interference (endogenous compounds of urine sample) behaviour is almost linear (see Figs. 3 and 4).

When the linear limits for the interference were evaluated, the GHPSAM calculated the analyte concentration free of bias error (Eq. (7)). In this equation the M values corresponds to standard solutions. The results are presented in Table 2. The relative error was acceptable in all instances since the highest errors were obtained in the region of detection, $2.1 \cdot 10^{-5}$–$7.02 \cdot 10^{-5}$ mol l^{-1}.

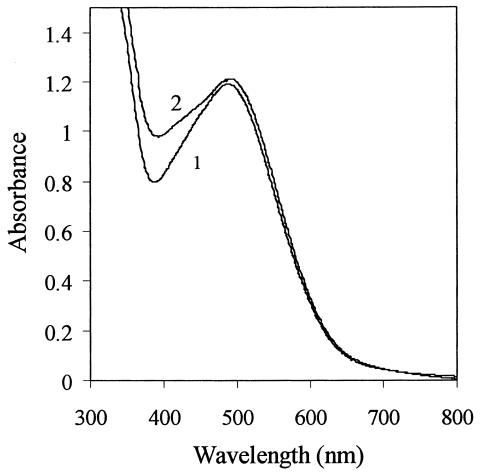

Fig. 3. Graph of absorbance, for urine spiked with the standard, versus λ (1) and graph of absorbance, obtained by adding the analytical signals of urine and cefotaxime standard, versus λ (2). The absorbance was recorded against an acetonitrile–water (1:1, v/v) blank at 514 nm. Conditions: NQS $7.1 \cdot 10^{-3}$ mol l^{-1}; cefotaxime $16.7 \cdot 10^{-5}$ mol l^{-1}; pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v).

The mean recoveries found using HPLC method [7] for the urine sample 2 was 98 ± 6 ($n = 24$). From this study, it can be concluded that the results obtained using both methods agree.

4. Conclusions

This study shows that using NQS as the derivatization reagent, previously retained in C_{18} columns, cefotaxime can be determined spectrophotometrically. The procedure used optimizes the reaction conditions in the C_{18} cartridges and is applied in this study to determine cefotaxime in pharmaceutical and urine samples. The generalized H-point standard additions method was used to measure cefotaxime in urine samples. This is a simple procedure which allows cephalosporins to be measured in a short analysis time. The volume of solvent

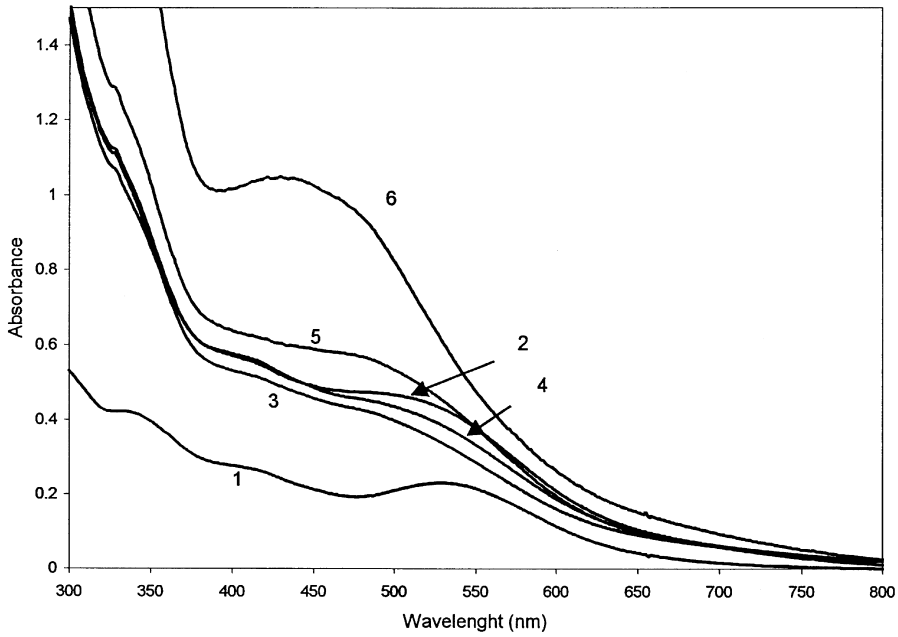

Fig. 4. Graph of absorbance, recorded against an acetonitrile–water (1:1, v/v) blank at 514 nm, versus λ for NQS reagent and for five different urines samples. (1) NQS reagent; (2–4) healthy adult; (5) child and (6) adolescent. Conditions: NQS $7.1 \cdot 10^{-3}$ mol l^{-1}; pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v).

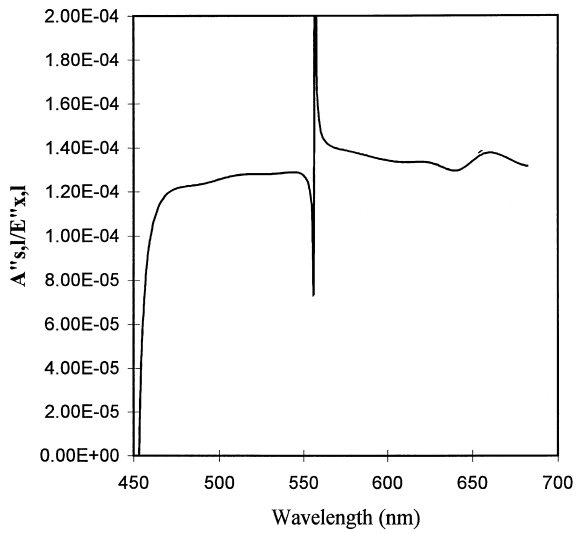

Fig. 5. $A''_{s,l}/\epsilon''_{x,l}$ versus λ_j plots for sample 13 urine 2 corresponding to a healthy adult according to Table 1. Conditions: NQS $7.1 \cdot 10^{-3}$ mol l^{-1}; Conditions: NQS $7.1 \cdot 10^{-3}$ mol l^{-1}; pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v), pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v).

employed is smaller than that required in a HPLC method. Sample clean-up and derivatization can be achieved in the same support.

Acknowledgements

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Appendix 1

Generalized H-point standard additions method [26]

We consider that the spectral behaviour for analyte X in the sample, with concentration C_X^O, can be described as:

$$A^0_{X,\,l} = C^0_X \times \epsilon_{X,\,l}\,;\; l \in [\lambda_1, \lambda_k] \qquad (1)$$

Table 1
C^{o} obtained for the $A''_{s,j}$ versus λ_j plots for the twenty-eight samples assayed

Sample no.	Cefotaxime ($mol\,l^{-1}$)·10^5)	Wavelength selected intervals	($C^0 \pm s_C^0$) found mean value ($mol\,l^{-1}$)·10^5)
Urine 1			
1	4.18	480–505	4.4±1.4
2	6.27	480–505	6±2
3	8.36	480–505	8.0±1.4
4	10.5	480–505	10±2
5	12.5	480–505	13±1.6
6	14.6	480–505	14.6±1.6
7	16.7	480–505	17.5±1.6
8	18.8	480–505	18.1±1.1
Urine 2			
9	4.18	480–510	4.9±1.1
10	6.27	480–510	5.0±1.4
11	8.36	480–510	8.4±1.8
12	10.5	480–510	10.9±1.7
13	12.5	480–510	13.1±1.4
14	14.6	480–510	15±1
15	16.7	480–510	17.3±1.1
Urine 3			
16	4.18	480–560	4.0±0.4
17	6.27	480–560	6.0±0.5
18	8.36	480–560	8.9±0.6
19	10.5	480–560	10.6±0.6
20	12.5	480–560	12.5±0.4
21	14.6	480–560	14.3±0.3
22	16.7	480–560	15.9±0.3
Urine 4			
23	4.18	530–560	4.5±0.8
24	6.27	530–560	6.6±0.6
25	8.36	530–560	8.3±0.3
26	10.5	530–560	11.0±0.7
27	12.5	530–560	12.9±0.7
28	14.6	530–560	14.8±0.7

Conditions: NQS $7.1\cdot10^{-3}$ $mol\,l^{-1}$; pH 10.5; temperature 25°C; and eluent, acetonitrile–water (1:1, v/v).

If the spectral behaviour of the unknown interferent, Y, can be described as a straight line in the spectral region selected, the resulting equation is:

$$A_{Y,l} = a + b \times \lambda_l \; ; \; l \in [\lambda_1, \lambda_k] \qquad (2)$$

For sample S, addition of analyte X at concentration C_X^O and interferent Y, the next expression for absorbance is:

$$A_{S,l} = A^0_{X,l} + A_{Y,l} = C^0_X \times \epsilon_{X,l} + a + b \times \lambda_l \qquad (3)$$

From Eq. (3) we can derive:

$$\frac{A''_{S,l}}{\epsilon''_{X,l}} = C^0_X \qquad (4)$$

Thus, the quotient of the second derivative spectrum of sample $A''_{S,l}$ and $\epsilon''_{X,l}$(calculated from the slopes of the calibration lines obtained from the second derivative spectra of the calibration or standard additions solutions) is a constant if the spectral behaviour of the unknown interferent Y is linear. The intervals at which this value could be considered constant were selected from plots of Eq. (4).

Let us suppose that using the procedure described above we have selected the wavelength interval [λ_1,

Table 2
Results obtained applying the generalized H-point standard additions method to twenty-eight samples

Sample no.	Concentration added ($mol\,l^{-1}\cdot10^5$)	Concentration found ($mol\,l^{-1}\cdot10^5$)	R.S.D. (%)	Relative error (%)
Urine 1				
1	4.18	4.9±0.5	9.72	−16.7
2	6.27	6.4±0.6	9.38	−1.95
3	8.36	8.5±0.6	7.06	−1.93
4	10.5	10.7±0.8	7.48	−1.98
5	12.5	13.7±0.9	6.57	−3.07
6	14.6	15.0±0.8	5.33	−3.07
7	16.7	18.0±0.8	4.44	−7.87
8	18.8	18.4±0.4	2.17	1.98
Urine 2				
9	4.18	5.2±0.2	3.85	−23.1
10	6.27	5.4±0.3	5.56	14.2
11	8.36	8.5±0.4	4.71	−1.43
12	10.5	10.7±0.4	3.74	−2.02
13	12.5	12.8±0.4	3.13	−2.25
14	14.6	14.5±0.4	2.76	−0.5
15	16.7	17.1±0.2	1.17	−2.57
Urine 3				
16	4.18	4.11±0.05	1.22	−1.74
17	6.27	6.38±0.03	0.47	−1.06
18	8.36	9.38±0.05	0.53	−12.2
19	10.5	11.10±0.05	0.45	5.99
20	12.5	13.10±0.04	0.31	4.42
21	14.6	15.00±0.04	0.27	2.94
22	16.7	16.60±0.04	0.24	−0.44
Urine 4				
23	4.18	4.7±0.3	6.38	12.3
24	6.27	6.7±0.3	4.48	5.56
25	8.36	8.6±0.2	2.33	3.18
26	10.5	11.1±0.2	1.80	6.13
27	12.5	13.0±0.2	1.54	3.75
28	14.6	15.0±0.2	1.33	2.41

The number of λ_m for the four wavelengths selected, intervals were 10, 17, 36 and 12, respectively.

λ_k] in which the spectral behaviour for the interferent can be considered linear. First of all, we must select a third wavelength, λ_m, belonging to the previously selected wavelength interval. It will allow us to correctly locate the H-point. The GHPSAM works with trios of wavelengths. We define two parameters, p and q, as:

$$p = \frac{\lambda_m - \lambda_1}{\lambda_k - \lambda_1} \quad (5)$$

$$q = \frac{\lambda_k - \lambda_m}{\lambda_k - \lambda_1} \quad (6)$$

The unbiased analyte concentration is calculated from the GHPSAM expression:

$$-C_H = \frac{q \times \Delta A_{S,(1,m)} - p \times \Delta A_{S,(m,k)}}{p \times (M_k - M_m) - q \times (M_m - M_1)} = \frac{q \times (A^0_{X,m} - A^0_{X,1}) - p \times (A^0_{X,k} - A^0_{X,m})}{p \times (M_k - M_m) - q \times (M_m - M_1)} = \frac{A^0_{X,m} - q \times A^0_{X,1} - p \times A^0_{X,k}}{q \times M_1 + p \times M_k - M_m} \quad (7)$$

where $\Delta A_{S,(1,m)}$ and $\Delta A_{S,(m,k)}$ are the absorbance increments of the sample at λ_1, λ_m and λ_m, λ_k,

respectively. The other symbols have the meaning defined in the text.

From these expressions we can optimize the values for λ_1, λ_m and λ_k to make the denominator in Eq. (7) larger, and obtain the most precise results. In addition, Eq. (7) can be used with *M* values obtained from standard addition lines or calibration graphs for pure analyte if matrix effects are known not to be present.

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