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Determination of triamterene by transitory retention in a continuous flow solid phase system with fluorimetric transduction

A. Domínguez-Vidal, P. Ortega-Barrales, A. Molina-Díaz *

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Paraje Las Lagunillas Ed. B-3, 23071 Jaen, Spain

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Abstract

A rapid and simple flow-through solid phase espectrofluorimetric system (sensor) is described in this paper for the determination of the diuretic triamterene at ng ml^{-1} level in physiological fluids and in pharmaceuticals. This sensor is based on the transitory retention of the analyte on the cationic ion-exchanger gel Sephadex SP C-25 placed into a quartz flow-cell in the detection zone itself of a spectrofluorimeter and the continuous monitorization of its intrinsic fluorescence. The spectrofluorimeter was tuned at 240 (excitation) and 440 nm (emission). A transitory signal was obtained because the carrier solution used also eluted the analyte from the sensing zone and no derivatization reactions were needed. Triamterene could be determined in the concentration ranges of 10–400 and 2.5–80 ng ml^{-1} with detection limits of 0.95 and 0.17 ng ml^{-1} for 300 and 1000 μl of sample volume, respectively. The relative standard deviations (RSD) for ten independent determinations and at three concentration levels of standards solutions were lower than 0.90 for 300 μl and 0.45 for 1000 μl. The RSDs for the determination of triamterene in serum samples and pharmaceuticals were lower than 3.3 and 2.6%, respectively. The method was satisfactorily applied to the determination of triamterene in human serum and pharmaceuticals.

Keywords: Diuretic; Triamterene determination; Fluorimetric flow-through sensor; Pharmaceuticals; Serum

1. Introduction

Diuretics are defined as substances that improve the renal excretion of water and electrolytes and are among the most extensively used drugs. Their action is based on interference with the mechanism of ionic transport along the complete length of the nephron. Triamterene (2,4,7-triamino-6-phenylpteridine) is a potassium sparing diuretic, which is widely used. It increases sodium excretion and reduces potassium elimination.

The chemical literature contains abundant references to methods for determining diuretics individually as well as in mixtures with other

* Corresponding author. Tel.: +34-953-012-147; fax: +34-953-012-141.
E-mail address: amolina@ujaen.es (A. Molina-Díaz).

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compounds, so, there are spectrophotometric [1–3], spectrofluorimetric [4], electrochemical [5–7] and high-performance liquid chromatographic methods with both spectrophotometric and fluorimetric detection [8–10]. Triamterene has mostly been determined by fluorescence spectrometry [11], capillary zone electrophoresis [12] and high-performance liquid chromatography [13,14].

Fluorimetric methods are extensively used in quantitative analysis because of their high sensitivity and selectivity in addition to their relative low cost; but they have not been extensively applied to the direct determination of fluorescent diuretics in mixtures because of the spectral overlapping. To resolve this question, synchronous [15] and derivative [16–18] fluorescence spectrometry is the most popular.

The use of a solid support to preconcentrate the species of interest (a native fluorophor or a fluorescence derivative from the analyte) improves substantially both sensitivity and selectivity of the fluorimeter solution methods [19,20].

The combination of flow-injection (FI) methodology with solid phase spectrofluorimetry join the advantages of unsegmented continuous flow analysis to those from solid phase spectrofluorimetry (the latter). The carrier stream is appropriately brought in contact with a solid surface placed just in the detection area of a spectrofluorimeter, so the solid phase on line preconcentration and separation of the fluorophor is performed simultaneously with the monitorization of its fluoresce signal. These continuous solid phase fluorimetric systems have been called fluorimetric flow-through sensors [21,22].

These sensing devices present some advantages such as sensitivity, selectivity, simplicity, low time required for the determination, in addition to a very low cost per analysis. They have made possible the direct determination of analytes in the presence of other foreign species also fluorescent, which show a strong spectral overlap without any prior separation. Nevertheless, although a few applications have been described in the field of pharmaceutical analysis, it should be noted that there is a scarcity of their applications to the analysis of biological fluids.

Here, we propose a flow-through spectrofluorimetric optosensor for the determination of triamterene in pharmaceuticals and biological fluids, serum, based on the measurement of its native fluorescence.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical reagent-grade chemicals by using doubly distilled water.

Stock standard solution of 100 mg l^{-1} of triamterene (Sigma, Buchs, Switzerland) was prepared by dissolving in the solvent mixture dimethyl sulfoxide/water (DMSO/water) (1:5 v/v). Solution was stable for at least 4 weeks at 4–5 °C. Work solutions were prepared fresh daily by appropriate dilution with doubly distilled water.

Sodium chloride solution of 0.08 M was prepared from Panreac (Barcelona, Spain) and adjusted to a pH = 2.0 with 0.1 N HCl (Panreac, Barcelona, Spain) and used as carrier/eluent solution.

Sephadex SP-C25 (Aldrich, Madrid, Spain) ion exchanger gel (40–120 μm; in H^+ form placed into a Hellma 176-QS flow-through cell) was used as sensing zone for the measurement of solid phase relative fluorescence intensity (RFI).

2.2. Apparatus

Spectrofluorimetric measurements were obtained with a Perkin–Elmer LS-50 luminescence spectrometer equipped with a xenon discharge lamp (20 kW), Monk-Gillieson monochromators, a Quantic Rhodamine 101 counter to correct the excitation spectra and a Gated photomultiplier. The luminescence spectrometer was interfaced with a Mitac MPC 3000F-386 microcomputer supplied with FL DATA MANAGER Software (from Perkin–Elmer) for spectral acquisition. Instrument excitation and emission slits were set at 10 and 20 nm, respectively, and the scan rate of the monochromators was 240 nm min^{-1}. The spec-

trometer was fitted with a Hellma 176-QS quartz flow cell (25 μl) with a light path length of 1.5 mm, packed with the solid support. The cell was blocked at the outlet with some glass wool to prevent displacement of the Sephadex particles by the carrier. The level of resin used was the sufficient one to cover all the detection area.

A four-channel Gilson Minipuls-3 peristaltic pump with rate selector, a Rheodyne Model 5041 injection valve and teflon tubing of 0.8 mm i.d. were also used.

2.3. Procedure

The working flow diagram is shown schematically in Fig. 1A. Three hundred micro litres (or 1000 μl) of an aqueous solution containing 10–400 ng ml^{-1} (or 2.5–80 ng ml^{-1}) of triamterene were inserted, by the injection valve, into the carrier solution (NaCl, $c_t = 0.08$ mol l^{-1}, pH = 2.0) and pumped at a flow rate of 1.98 ml min^{-1} to the flow cell where triamterene was transitorily retained on the support. The fluorescence emission intensity was measured continuously at 440 nm (20 nm slit-width) using an excitation wavelength of 240 nm (10 nm slit-width). The carrier itself then elutes and regenerates the active microzone of the sensor thus restoring the baseline.

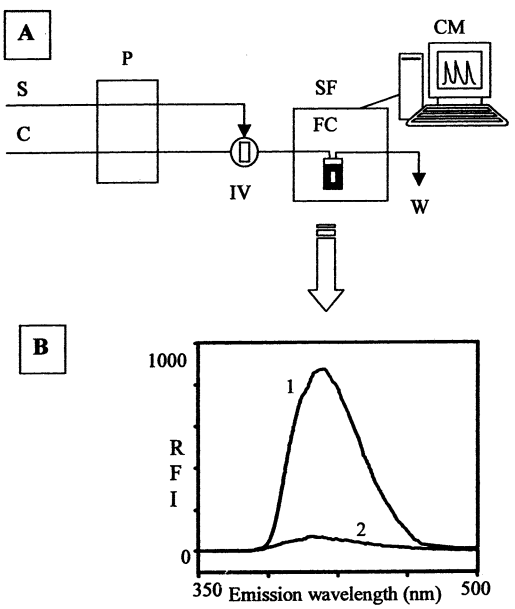

Fig. 1. Schematic diagram of the flow system: P, peristaltic pump; C, carrier; S, sample; IV, injection valve; SF, spectrofluorimetric detector; FC, flow cell; CM, computer; W, waste. (B) Scans of the same concentration of triamterene sorbed on Sephadex SP C-25 (1) and in solution (2).

2.4. Sample preparation

2.4.1. Pharmaceuticals

Tablets powder of triamterene (from 20 tablets) were accurately weighed and dissolved in the appropriate amount of the solvent mixture, 20% (v/v) DMSO/water. Then solutions were filtered through a 0.45 μm membrane filter (Millipore) to remove any remaining insoluble matter, and diluted to appropriate volume with the same solvent mixture.

2.4.2. Human serum

The *Ciudad de Jaén Hospital* (Jaén, Spain) kindly supplied the serum samples (obtained after coagulation and further centrifugation at 3000 rpm for 5 min). The samples were used directly after appropriate dilution with 20% DMSO/water for the analysis.

3. Results and discussion

3.1. Spectral characteristics

Due to its cationic nature at low pH values, triamterene (a native fluorophor) is retained on Sephadex SP C-25 cation exchanger gel. Fluorescence spectra of triamterene, both in aqueous solution and on the Sephadex SP-C25 resin, were obtained. The analytical response is about 30 times higher than that obtained in solution by a conventional FI system under the same working conditions (Fig. 1B).

In both cases excitation and emission spectrum RFI maxima were located at 240 and 440 nm, respectively. Fig. 2 shows the contour map of triamterene.

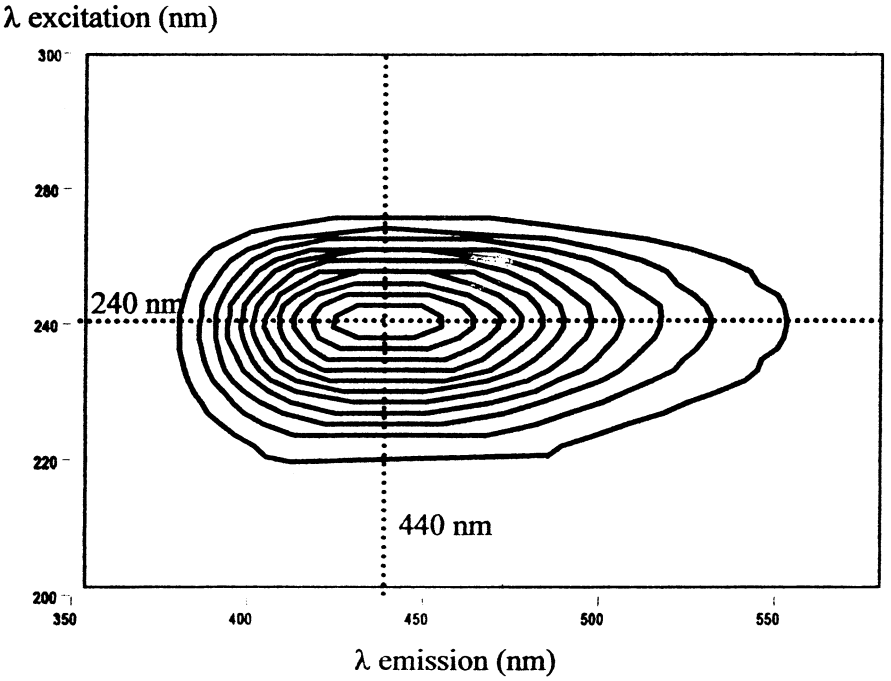

Fig. 2. Contour plot of triamterene made in solution.

3.2. Influence of the pH and concentration of the carrier solution and sample

The influence of the carrier solution pH on the retention–elution of triamterene on the solid support was studied in the range 1.9–11.3 using hydrochloric acid and sodium hydroxide for adjustment. The results showed a minimum decrease in the analytical signal with increasing pH, so pH = 2.00 was chosen as optimum because of its easily preparation from adjustment of deionised water with HCl.

Several electrolyte solutions at pH = 2 were tested as carrier (sodium chloride, potassium chloride, sodium nitrate and sodium sulphate), the most suitable carrier solution was NaCl and the influence of its concentration was studied by varying it from 0.01 to 0.4 M. As can be seen in Fig. 3A, a 0.08 M concentration produced the highest analytical signal also eluting completely the analyte from the sensing zone in a reasonable short time. Therefore, no eluent solution different from the carrier was required, thus allowing a high sampling frequency and a higher lifetime for the sensing zone.

The sample pH value did not influence the analytical signal when its value was varied from 2 to 10; so, the adjustment of the sample pH was not necessary.

3.3. Influence of the emission and excitation slit width

The influence of the excitation slit width on the signal was scarcely significant while the signal strongly increased as the emission width slit increased (Fig. 3B), 10 nm for excitation and 20 nm for emission gave the maximum RFI signal so they were chosen as better for the sensor.

3.4. Influence of the flow rate

Fig. 3C shows the influence of the flow rate on the RFI of triamterene fixed on the resin as well as on the sampling frequency. As can be seen, the RFI and the elution time decreased when flow

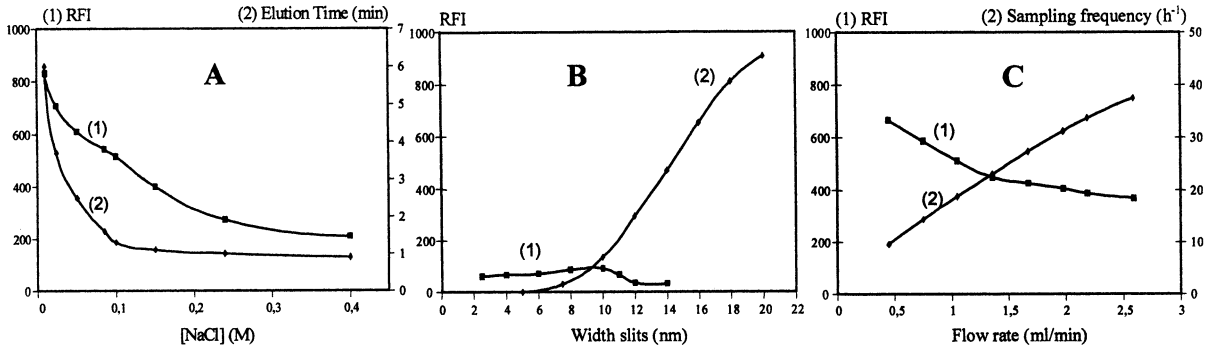

Fig. 3. Influence of some experimental variables; (A) influence of the carrier concentration, NaCl (M), on RFI (1) and elution time (2); (B) influence of the excitation (1) and emission (2) slits width on RFI; and (C) influence of the flow-rate on RFI (1) and sampling frequency (2). Concentration of triamterene was 250 ng ml^{-1} and injection volume of 300 μl in all the cases.

rate was increased from 0.5 to 2.6 ml min^{-1}. The result is similar to that found in other flow-through spectroscopic sensor [23]: the amount retained on the sensing zone obviously decreases as the flow rate increases as the analyte cannot be retained on the solid support from the sample plug in an instantaneous way.

A flow rate of 2.0 ml min^{-1} was selected as a compromise between the sensitivity and the throughput (the signal decreasing 39 against 78% in the throughput, in comparison with those for 0.5 ml min^{-1}).

3.5. Influence of sample volume

The increase in analytical response as sample volume injected increased could be exploited in order to obtain a significant increase in sensitivity by simply constructing two different calibration lines. The analytical signal was linearly related to the sample volume injected from 40 to 2000 μl according to the experimental equation: $RFI = 110.3 + 0.595v$ ($r = 0.9982$; v, μl; triamterene concentration of 0.1 μg ml^{-1}). Beyond 2000 μl the increase in analytical response was lower.

The selection of the injection volume is related to the concentration of the analyte in the samples for analysis, so, in physiological fluids more sensitive methods are needed because of the lower concentration of triamterene and an injection volume of 1000 μl was chosen. In the case of pharmaceuticals the concentrations are higher, so higher sensitivity is not necessary and it is preferred to improve the sampling frequency. Three hundred micro litres of injection volume was chosen.

3.6. Features of the proposed sensor

Analytical figures of merit of the proposed sensor for the two different injection volumes (300 and 1000 μl) are given in Table 1. The calibration of the sensor was carried with seven standard solutions (excluding blank value) for the two chosen volumes.

Table 1
Figures of merit

Parameter	Volume of sample loop (μl)	
	300	1000
Calibration graph		
Intercept	−1.770	−0.044
Slope (ml ng^{-1})	2.155	11.066
Correlation coefficient	0.999	0.999
Linear dynamic range (ng ml^{-1})	10–400	2.5–80
Detection limit (ng ml^{-1})	0.95	0.17
Quantification limit (ng ml^{-1})	3.16	0.58
RSD (%) ($n = 10$)	0.87 (10)	0.42 (5)
concentration level	0.33 (280)	0.24 (65)
(ng ml^{-1})	0.20 (400)	0.15 (80)
Sampling frequency (h^{-1})	30	25

Table 2
Interference study (determination of 60 ng ml^{-1} of triamterene)

Foreign species	Tolerated interferent/analyte (w/w) ratio
Uric acid, urea	>10 000[a]
Lactose, glucose, saccharose	>1000[a]
Ascorbic acid	>1000[a]
Furosemide	40

[a] Maximum ratio tested.

The precision of the method, expressed as relative standard deviation (RSD) ($n = 10$) was studied with a series of ten standards having concentrations of 10, 280 and 400 ng ml^{-1} for 300 μl of sample volume and 5, 65 and 80 ng ml^{-1} for 1000 μl.

The precision of the method in the analysis of serum samples was established following the guidelines from conference on Analytical Methods Validation [24]. Six serum sources, each spiked at four different level concentrations (10, 20, 40 and 60 ng ml^{-1}) were used. In each case six replicate determinations were carried out. The RSDs percentage found for any serum sample were lower than 3.3% for the four concentrations tested.

The RSDs percentage related to the reproducibility between matrices (calculated from the average values found for the six replicates of each concentration used in the six different serum samples) ranged from 5.1 (10 ng ml^{-1}) to 4.3 (60 ng ml^{-1}).

Detection and quantification limits were also calculated by using the criteria 3σ [25] and 10σ [26], respectively, for both calibrations (Table 2).

3.7. Influence of foreign species

The interference of those species commonly accompanying triamterene in pharmaceuticals and serum was studied. The effect of these foreign species on the determination of triamterene by the proposed sensor was carried out by adding known amounts of foreign species to a triamterene solution of 60 ng ml^{-1} and using a sample volume of 1000 μl. Tolerance was defined as the amount of foreign species that produced an error not exceeding ± 3% in the determination of the analyte.

The procedure proposed here shows a high tolerance level to other species frequently found along with triamterene. This effect is mainly due to the retention of the triamterene on the resin and the consequent separation from the matrix, which increases selectivity.

3.8. Analytical applications

The proposed sensor was applied to the determination of triamterene in:

(A) Pharmaceutical preparations and,
(B) Physiological fluids: six samples of serum.

3.8.1. Pharmaceuticals

The procedure was used with a sample loop of 300 μl in the two pharmaceuticals found in the Spanish Pharmacopoeia. Results are shown in Table 3. The values show a good agreement with those given by the manufacturers. A recovery study was performed by adding three different amounts of triamterene to each drug. Recovery results range was 96.3–99.1%.

Table 3
Determination and recovery study of triamterene hydrochloride in pharmaceutical preparations

Triniagar[a]	
Stated content (mg)	Found[b] (mg ± SD)
50	48.9 ± 0.5
Added (mg/unit)	Recovery[b] ± SD (%)
10	94.9 ± 0.3
25	96.3 ± 0.9
50	97.2 ± 0.5
Salidur[c]	
Stated content (mg)	Found[b] (mg ± SD)
25	25.8 ± 0.7
Added (mg/unit)	Recovery[b] ± SD (%)
5	99.1 ± 0.5
10	98.7 ± 0.5
20	96.3 ± 0.6

[a] Tablets containing: triamterene chlorhidrate, 50 mg; mebuticide, 50 mg; lactose and other excipients.
[b] Data are average of three determinations.
[c] Tablets containing: triamterene chlorhidrate, 25 mg; furosemide, 77.35 mg; excipients.

Table 4
Recovery study of triamterene hydrochloride in physiological fluids

Sample	Serum	
	Added ($\mu g\,ml^{-1}$)	Recovery[a] $\pm \sigma_R$ (%)
1	10	104.2 ± 0.3
	35	100.6 ± 0.1
	60	100.7 ± 0.5
2	20	97.4 ± 0.3
	50	100.1 ± 0.4
	70	99.7 ± 0.3
3	15	98.2 ± 0.4
	25	99.9 ± 0.2
	55	100.1 ± 0.1
4	20	99.4 ± 0.6
	40	95.6 ± 0.2
	60	100.2 ± 0.4
5	30	102.1 ± 0.3
	45	96.6 ± 0.1
	70	97.5 ± 0.6
6	5	102.1 ± 0.3
	16	96.6 ± 0.1
	40	97.5 ± 0.6

[a] Data are average of three determinations.

3.8.2. Physiological fluid: serum

The samples were found to be free from triamterene, so they were prepared by spiking with known amounts of the analyte, from 0.3 to 0.9 $\mu g\,ml^{-1}$. These amounts were chosen because the levels of triamterene in serum are 60–90% of the swallowed, so from the pharmaceuticals cited, the concentrations levels would be in the 3–9 $\mu g\,ml^{-1}$ range for serum. The determination of these samples was performed from an adequate aliquot by standard addition method due to the high matrix effect (showed by the slope's ratios *addition calibration graph*/*standard calibration graph*: 0.6–1.2). The recovery results range was 95.3–104.9% thus confirming the validity of the proposed sensor (Table 4).

4. Conclusions

The continuous flow-through spectrofluorimetric sensor here developed shows the viability of an automatic continuous analysis for the determination of triamterene in pharmaceuticals and serum samples. It is based on the transitory retention of triamterene on an active solid support and the measurement of its native fluorescence without any derivatizing reaction. The results show a very interesting improvement in sensitivity, selectivity, simplicity and speed, all of them due to the preconcentration of the analyte on the cation exchanger gel, thus preventing the interference from other non-cationic species. Moreover, these features in combination with an FIA system in which the carrier itself regenerates the active microzone make the sensor a cheap, continuous, simple and reusable sensing device suitable for the determination of triamterene.

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