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Determination of betamethasone-17-valerate in betason and urine samples using polyurethane foam modified with bromophenol blue

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Abstract

A simple and sensitive spectrophotometric determination of betamethasone valerate (BV) in pharmaceutical and urine samples using on-line preconcentration system. A minicolumn packed with bromophenol blue grafted polyurethane foam (BPB-PUF) has been used for the preconcentration of BV. The optimum preconcentration conditions were based on the extraction of the BV at pH 9.5, preconcentration time 90s and flow rate 1.0 mL min^{-1}. Desorption of BV was effected by passing 300 μL, 0.50 mol L^{-1} hydrochloric acid as eluent. The preconcentration system provides a linearity range 0.05 – 30 μg mL^{-1}. The limits of detection and quantification are 0.02 mg L^{-1} and 0.07 mg L^{-1}, respectively. The developed approach provided an enrichment factor 74 fold within the concentration range 2.0 - 15.0 mg L^{-1}. Method precision expressed as relative standard deviation (RSD) was found to be 1.9 % for six replicate measurements at concentration level 5.0 mg L^{-1}. The method was successfully applied to the determination of BV in pharmaceutical and spiked urine samples with recovery ranged from 101 to 104% and RSDs less than 4.5%.

Keywords

Betamethasone valerate; Preconcentration; Bromophenol blue; Polyurethane foam.

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INTRODUCTION

Betamethasone valerate (BV), 9α-fluoro-16β-methylpred-nisolone, is a semi-synthetic active pharmaceutical ingredient or an intermediate which belongs to the family of glucocorticoid steroids. It is used as strong anti-inflammatory, Immunosuppressive agent to stimulate fetal lung maturation, to decrease the incidence and mortality from intracranial hemorrhage in premature infants, and topical pharmaceutical creams to relieve skin irritation[1]. Determination of topical dosage like BV suffers from the interference due to formulation and preservatives exist in these pharmaceuticals. Liquid chromatography is the most common technique used for the determination of betamethasones[2]. Typically, high performance liquid chromatography (HPLC) tandem to mass spectrometry[3] either with electrospray ionization[4] or with isotope dilution[5] are now widely employed. Furthermore, comprehensive screening approach was applied to the determination of acidic and neutral betamethasone valerate in equine plasma by liquid

chromatography-tandem mass spectrometry[6].

In literature, a short precolumn containing octadecylsilane based sorbent mounted into the sample loop position of an injection valve was used as the primary clean-up step followed by diode-array UV detector for quantitative analysis of BV[7]. Solid phase extraction (SPE) coupled to spectrometry has been successfully applied to the determination of organic[8] and inorganic[9] analytes using several types of solid sorbents, such as amberlite resin[10], activated carbon[11], silica gel[12] and polyurethane foam[13]. The literature reviews cite few works about the use of PUF for the determination of organic substances carrying out an on-line elution directly to the UV detector[14]. In the present work, BPB is chemically grafted into the skeleton of PUF to be used as a new sorbent capable to form solid phase extractive ion-pair with BV in on-line preconcentration/separation mode. The proposed procedure will be validated by the fast determination of BV in pharmaceutical and urine samples.

MATERIAL & METHODS

Reagents

All chemicals were analytical-reagent grade (Merck, Darmstadt, Germany) and were used without previous purification. The glassware was washed with deionized water and dried. Stock solution of BV with a concentration of 1000.0 μg mL^{-1} was prepared by dissolving an appropriate amount of BV (From National Organization for Drug Control and Research (Egypt)) in ethanol. The chemicals used in synthesis of polyether type PUF including polyol or polyethylene oxide-polyethylene glycol, triethanolamine, stannous octoate, 2,4-toluene diisocyanate (TDI) were obtained from Safa Foam, a local company for foam manufacture, industrial zone, New Damietta, Egypt. Bromophenol blue was purchased from Aldrich (Milwaukee, USA). Pharmaceutical sample (Betason tablet) was purchased from Elnasr Company, Cairo, Egypt. The pH of the final solutions was adjusted by suitable addition of a 10% (m/v) sodium hydroxide solution and an appropriate buffer solution, and the mixture was finally diluted to 25 mL by double deionized water.

Apparatus

All spectrophotometric measurements were performed by use of UV - 1601 Shimadzu double beam spectrophotometer (Kyoto, Japan). pH was measured with a pH meter model GP353 equipped with an EDT combined glass electrode with an accuracy ± 0.01. A mechanical shaker (SL 350NUve scan. Akyurt Ankara – Turkey) throughout operated at 200 $Umin^{-1}$ was used to shake solutions. Doubly distillations occurred by Hamilton laboratory glass instrument (Europe House, Sandwich, England). All experiments were done at room temperature.

Preparation of bromo phenol blue poly urethane foam (BPB-PUF)

An accurately weighed 20 ± 0.01g liquid polyol was mixed to 0.20 g triethanolamine, 1.0 mL doubly distilled water, and 0.02 g BPB. The mixture was stirred well till complete homogeneity of the mixture indicated by uniform distribution of the dye blue color. After this, 0.04 g stannous octoate was added to the mixture and stirred for about 3 min, then; TDI (13.0 g) is poured into the mixture in a single addition under vigorous stirring in polyethylene box lined with paper. The liquid is left for curing at room temperature and the solid material was cut into a small cylindrical plugs with metallic slicer, packed into a polyethylene tube and integrated to the on-line manifold depicted in Figure 1.

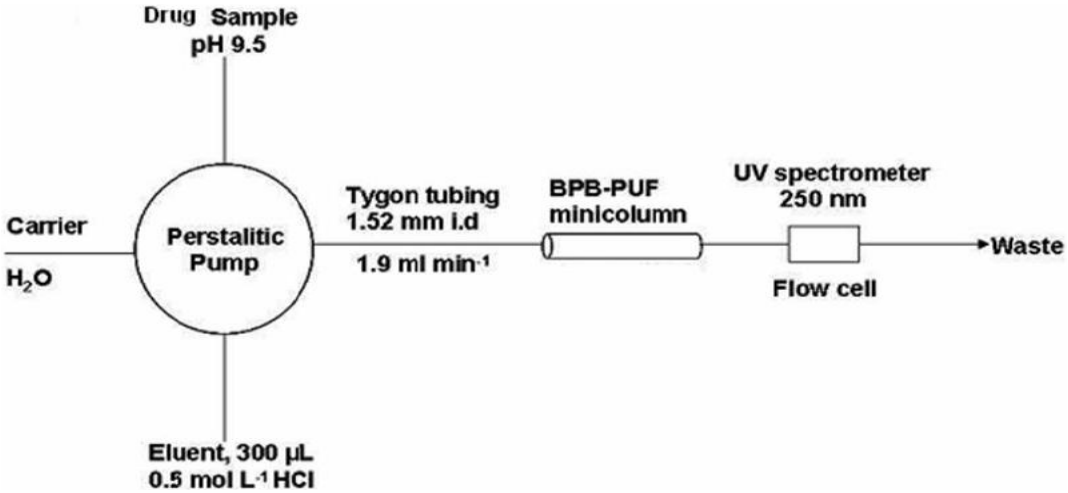

Figure 1 : On-line preconcentration manifold for the determination of BV.

General procedure

Preconditioning of the minicolumn prior to its use was done by passing 5 mL of buffer through the BPB-PUF in order to be ready for extraction by activating the adsorption sites and solvation of the functional groups. Afterwards, about 20 mL of doubly distilled water was pumped through the minicolumn, at flow rate 5 mL min^{-1}, to wash and to eliminate the excess of buffer. This step was performed daily before starting the system operation. Preconcentration of BV (Figure 2) was performed using the manifold by passing the BV sample through the minicolumn at a flow rate of 1.0 mL min^{-1} for 90s preconcentration time followed by passing doubly distilled water carrier till the absorbance baseline has been restored. Then, 300 µL of 0.5 mol L^{-1} hydrochloric acid solution was injected into the carrier stream at 1.0 mL min^{-1} to displace the analyte from the solid sorbent and the signals were recorded as peak height absorbance at 250 nm. This procedure was adopted for both of pure, pharmaceutical and real sample.

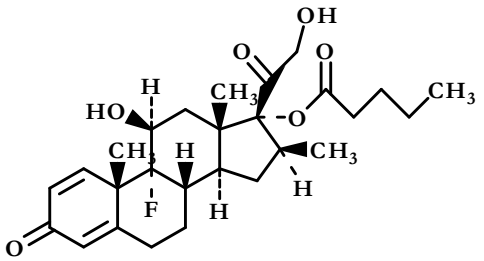

Figure 2 : Chemical structure of BV compound.

Sample preparation

An accurately weighed 2.0 mg Betason sample was dissolved in the least amount of methanol then the pH of solution was adjusted to pH 9.5 and completed up to 100 mL with doubly distilled water. BV free human urine sample was collected in clean polyethylene bottles. The sample was divided into 5 mL portions; each portion was spiked with 1.2, 2.0 or 3.0 mg from BV and adjusted to pH 9.5. The peak height for the recovered BV was measured by UV detection at 250 nm.

RESULTS AND DISCUSSION

Characterization and stability of Bromo phenol blue - Poly urethane foam

The infrared study was used to confirm the expected grafting BPB to the backbone of PUF. The IR spectrum of BPB-PUF showed several additional and characteristic bands that do not appear in the spectrum of untreated PUF. The absorption band observed at 616 cm^{-1} could be assigned to the C–Br stretching. Also, a broad absorption band 3650-3100 cm^{-1} which might be characteristic for phenolic OH and SO_3H groups. The characteristic peak observed at 1413 cm^{-1} could be assigned to stretching frequency for the CS group in BPB reagent indicating its binding to PUF skeleton.

Stoichiometric of Bromo phenol blue - Poly urethane foam

The chemical stability of BPB-PUF sorbent was investigated by testing the leaching out of the dye by several polar organic and inorganic solvents. For this purpose, the BPB-PUF was immersed in the test solvent for 1h. and after each treatment; the concentration of BPB in the testing solvent was measured spectrophotometry. Polar organic solvents namely methanol, ethanol, acetone were studied. Inorganic solvents including 2 mol L^{-1} hydrochloric and 1 mol L^{-1} acetic acids. Furthermore, the influence of alkaline media such as 4 mol L^{-1} sodium hydroxide and 1 mol L^{-1} ammonia solutions were tested. The results indicated no significant effect of all examined solvents on the retained dye since nearly zero absorbance for the BPB dye was detected in the effluent of each tested solvent which ensures good chemical stability of BPB-PUF. Only a change in the color of the grafted foam from blue to yellow was observed by changing the medium from alkaline to acidic, respectively. The solvent extraction might be the most probable mechanism of BV sorption on to grafted PUF. The mechanism based on membrane-like structure of the foam together with its efficient sorption properties offered higher concentrating ability. The BV containing which able to form hydrogen bond with urethane NH, terminal NH_2, ether oxygen CH_2-O-CH_2 and with SO_3 of grafted BPB[15].

Optimization of the on-line preconcentration procedure

Effect of sample pH

The influence of varying pH on the extraction of BV by BPB-PUF was studied in range 2.0-11.0. The samples were adjusted either by potassium hydrogen phthalate/ hydrochloric acid, acetate buffer and ammonium buffer in the pH ranges 2-5, 6-7 and 8-11, respectively. The absorbance signal for the recovered BV was found to be relatively low and constant within the pH range 4-7. At pH ≥ 8, the absorbance linearly increased. This confirms the BV could not be able to form ion pair complex with BPB in neutral or faint acidic media. However, in basic medium the BV were extensively transferred to the solid phase. Finally, sample solution at pH 9.5 adjusted with NH_4OH/

H^+ buffer was recommended in the subsequent experiments (Figure 3).

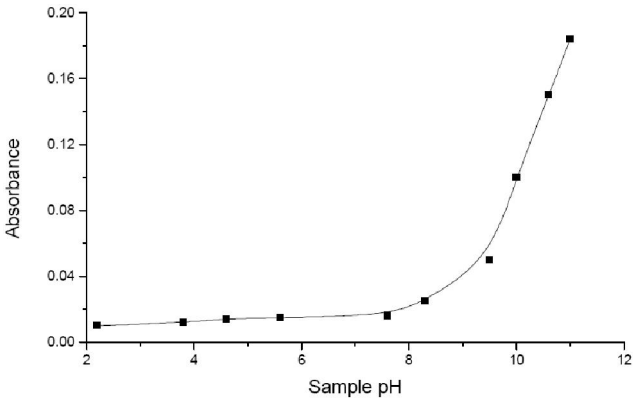

Figure 3 : Effect of the sample pH on the peak height absorbance of BV (5.0 mg L^{-1}).

Preconcentration time

The preconcentration time was investigated in the range between 10 and 200s. The absorbance peak height was increased linearly in the range 60-200s. However, at lower preconcentration times than 60s, another straight line was obtained but with lower slope (Figure 4). Therefore, a preconcentration time up to 200s could be applied in the preconcentration step which would improve the detection limit of the procedure since much amount from the BV would be retained in the minicolumn. Finally, a 150s preconcentration time was selected in the subsequent experiments as a compromise between method sensitivity, sample consumption, and effective sample throughput.

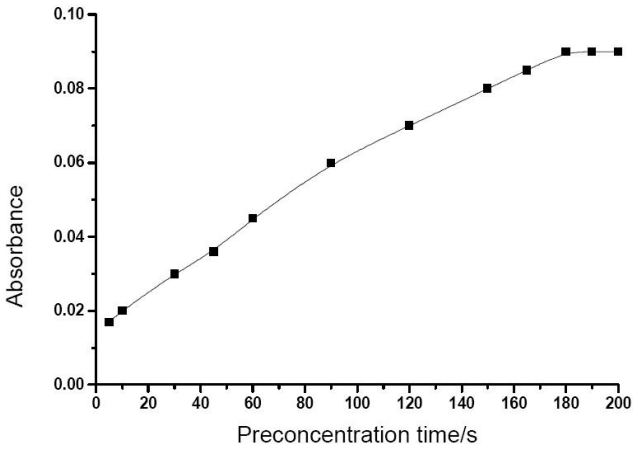

Figure 4 : Effect of the preconcentration time on absorbance of BV (5.0 mgL^{-1}).

Sample flow rate

The retention of sorbent depends on the flow rate of the sample solution[16]. Thus, the effect of flow rates of the sample and elution solutions on the retention and uptake of is an important parameter, since it not only affects the recovery of the analyte, but also controls the time of analysis. It is also expected that sample solutions can be passed through column at a higher flow rate without sacrificing the recoveries. So, sample flow rate investigated under the optimum conditions to obtain quantitative retention and elution of the BV. The influence of sample flow rate was examined to the proposed preconcentration system within the range from 0.50 to 2.2 mL min^{-1} as indicated in Figure 5. The absorbance signal of BV was found strongly influenced by the flow rate of sample since the recovered absorbance signal was gradually decreased with increase flow rate. Maximum absorbance was obtained when the flow rate is between 0.8 and 1.0 mL min^{-1}. This implies the contact time between BV and BPB is sufficient. Indeed, the working range of sample flow rate is relatively short which indicates high sensitivity of the formed ion-pair complex to the contact time. Hence, 1.0 mL min^{-1} was recommended so that to achieve high sensitivity and moderate sample consumption.

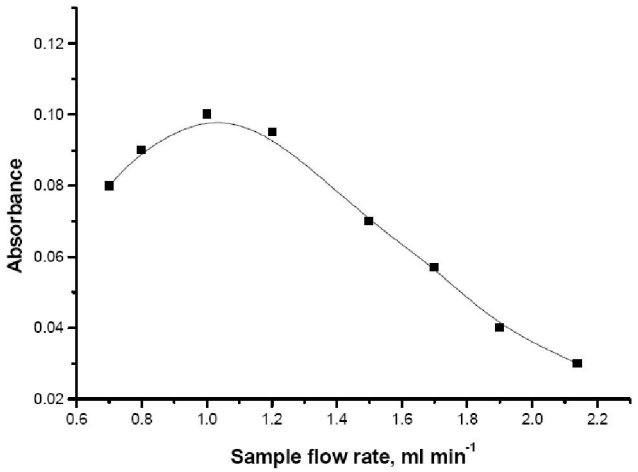

Figure 5 : Dependence of absorbance of BV (5.0 mgL^{-1}) on the flow rate of the sample.

Eluent study

Eluent nature was carefully investigated since it affects both analytical efficiency and throughput. Hydrochloric acid was recommended as sorbent since it would rapidly alter the pH of the medium thus impose fast decomposition of the ion-pair complex. The proposed hydrochloric acid eluent was examined in the concentration range from 0.01 to 1.0 mol L^{-1}. Best absorbance signal was obtained at acid concentration 0.5 mol L^{-1}. Lower concentrations than 0.5 mol L^{-1} would produce too low signals which might be due to insufficient amount from H^+ necessary to neutralize OH^- in the buffer. At increased acid concentration than 0.5 mol L^{-1}, the absorbance signals were decreased which might be attributed to the synergistic effect of the chloride ion due to high ionic strength of the medium which might resist an easy removal of the BV mol-

ecules from the sorbent. Finally, a 0.5 mol L^{-1} acid concentration was chosen as suitable eluent in the further investigations.

The volume of the eluent solution was also optimized because it affects both of the height and broadening of the analytical signal and consequently the recovery data. The absorbance signals of BV was maximum and constant when the eluent volume in the range from 200 to 300 μL. The larger eluent volumes than 300 μL have led to significant reduction in absorbance which can be due to diffusion of the BV over the elute stream to give broad peaks with lower intensities. The suitable volume of HCl required ensuring quantitative elution of the retained BV was set at 300 μL and in all subsequent studies it was applied in the elution step.

Break through capacity

The dynamic capacity of BPB-PUF towards BV was estimated from the breakthrough curve. When a standard solution of BV, prepared at concentration 2 mg L^{-1} and pH 9.5, was continually passed through the minicolumn at flow rate 1.0 mL min^{-1}. A breakthrough curve was obtained and the capacity was calculated from the breakthrough point which represents the first detection of BV in the effluent and the achievement of maximum retention capacity of the sorbent under such flow conditions. The obtained dynamic capacity was 0.55 mg g^{-1} (1.15 μmol g^{-1}) and this value is well above the content of BV in analyzed portion from pharmaceutical and urine samples.

Analytical performance

The plot of peak height absorbance against BV concentration has afforded a linear analytical curve in the range from 0.05-25.0 mg L^{-1} at flow rate 1 mL min^{-1}, as shown in Figure 6. The calibration equation could be represented by:

$$A = 1.017\ C + 0.001$$

Where A and C are the absorbance peak height and BV concentration in mg L^{-1}, respectively. The correlation coefficient $R^2 = 0.969$, Furthermore, the run time for each analysis was about 5 min which provides a sample throughput 12. The limit of detection (LOD) was calculated as three times the standard deviation which is 0.02 for standard solutions of BV and the limit of Quantification (LOQ) calculated as ten times the standard deviation and found to be 0.07. The calibration graphs were constructed using standard solutions of BV at these optimum conditions a linear relationships existed between the absorbance and concentration of the BV in concentration range listed in TABLE 1. The correlation coefficient, slope, intercept standard deviation of slope and standard deviation of intercept of calibration data for BV. The reproducibility of the methods were determined by running six replicate samples each of 5.0 μg mL^{-1} of the BV, the relative standard deviation was calculated as shown in TABLE 1.

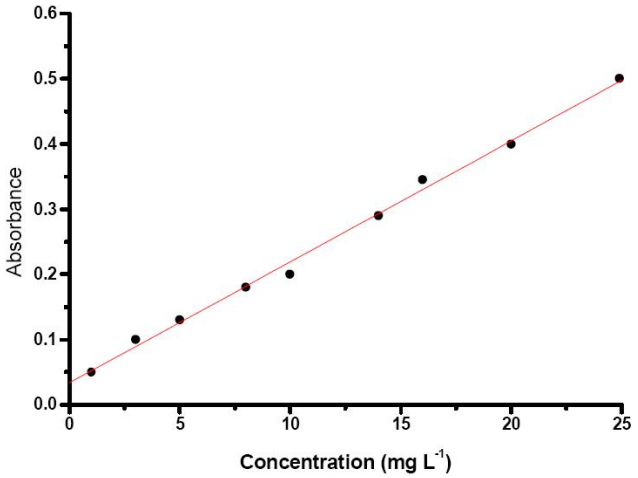

Figure 6 : Analytical performance curve of BV with pH 9.5 preconcentration time 150s measured at 250 nm.

TABLE 1 : Optical characteristic and precision data of on-line preconcentration of BV using BPB-PUF sorbent

Features	BV
pH	9.5
Lower Detection-limits(mg L^{-1})	0.02
Standard deviation (SD)	0.013
Quantification limit(mg L^{-1})	0.07
Break Through Capacity(mg g^{-1})	19.01
RSD (%)	1.188
Beer's law limits (mg L^{-1})	0.05-25
Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$) x 10^{-5}	6.229
Correlation Coefficient (r^2)	0.969

The accuracy of the proposed analytical method was investigated in order to governance its feasibility in analytical applications. Intra-day precision of the method was determined by running six replicate determinations (n = 6) at 3, 5.0 and 10 mg L^{-1} and the relative standard deviation of the mean was taken as a measure of the precision, as summarized at TABLE 2. Furthermore, accuracy, expressed as the mean relative error RE, was evaluated from the ratio of the difference between the measured and actual concentrations to the actual concentration of the BV in the samples[17].

Fixed amount (5 mg L^{-1}) of BV was passed through

the column before adjusted to recommended pH. The experiment was repeated six times and the uptake capacity was calculated. The resulted RSD value were in the range 0.9 – 1.2 which are acceptable for applying BPB-PUF in the determination of the BV in biological and pharmaceutical samples. The RSD values were found to be 1.9 % BV which confirms intra-day assay error was less than 3%. Also, the RE was found to be +1.4 % BV concentration 5.0 mg L^{-1} which are considered quite good.

TABLE 2 : Determination of BV in betason tablets applying standard addition technique using BPB-PUF

Drug	Taken mg	Found mg	Recovery (%)	RSD (%)	RE[(a)] (%)
Betason	0.15	0.153	102.0	0.16	0.06
(BV)	0.25	0.252	100.8	0.46	0.17
	0.50	0.485	97.0	1.33	0.47

(a): % Relative Error ({SD/SQRT(n)}*100

Interference study

To emphasis the feasibility of the method for pharmaceutical and urine analysis, the effects of some coexisting species found as major components in real samples were investigated. Organic substances that often accompany betamethasone valerate in pharmaceutical preparations and commonly encountered pharmaceutical additives and excipients such as glucose, lactose, starch, glycine, fructose, ammonium sulfamate and some inorganic salts were examined. The tolerance levels were found relatively high (≥500 mg L^{-1}) and corresponding recovery values were quantitative (97 – 105 %) as shown in TABLE 3. Any deviation of ±5% or more from the standard absorbance value was taken as interference.

TABLE 3 : Recovery of betamethasone valerate (5 mg L^{-1}) in the presence of recipients and other substances:

Foreign Substance	Tolerance level, mg L^{-1}	Recovery (%)
Glucose	500	101
Glycine	600	96
Histidine	> 1000	101
Glutamine	> 2000	99
Starch	> 2000	101
Urea	2000	101
Ammonium sulphamate	4000	100
NaCl	5000	95
KCl	2000	100
$CaCl_2$	5000	98

Applications

The validity of proposed procedures was tested by determining BV in Betason tablet. The found concentration of BV in dosage forms was calculated by matching the absorbance peak height of the sample to the corresponding concentration in the established analytical curve. Recoveries of BV from test samples were determined in triplicates by comparing the found with the taken amount. Different levels of BV amounts (e.g. 3.0, 5.0 and 10.0 μg) were taken into 3.0 mL test samples. Average recovery in the range 101 - 104%, the RSD % vary from 0.2 to 4.5 % and relative error (RE) from 0.8 to 4.0 %. Furthermore, the developed preconcentration manifold has been applied to the determination of BV in spiked urine. The within-day recoveries and relative standard deviations (RSDs) of spiked BV in urine at the levels of 1.2, 2, and 3 mg L^{-1} are summarized in TABLE 4. The recoveries obtained by analyzing the spiked samples were satisfactory (recovery ≥ 89%).

TABLE 4 : Recovery data for the determination of BV in 5 mL spiked urine sample (n=3).

Spiked level of BV mg L^{-1}	Found (mean ± SD) mg L^{-1}	Recovery (%)
1.2	1.07 ± 0.03	89
2.0	1.83 ± 0.10	92
3.0	2.74 ± 0.08	91

CONCLUSIONS

A fast, simple and reliable continuous method has been developed for quantification of betamethasone valerate in pharmaceutical and urine samples. The proposed BPB-PUF sorbent provided fast sorption/desorption properties and allowed the on-line determination of the BV. The sample is injected into the preconcentration minicolumn and the eluted BV is directed to the flow cell in UV-Vis spectrophotometer in closed cycle to minimize the possible contamination. The extensive sample preparation is not needed which allows easy and rapid method development. The run-time for each sample was 5 min, adequate sensitivity and selectivity was achieved with a traditional UV detector. The LOD attained in this study allow quantification of BV in pharmaceutical preparations and urine. The method is suitable for routine tests to control the release profiles of BV with sufficient accuracy.

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