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Determination of verapamil by adsorptive stripping voltammetry in urine and pharmaceutical formulations☆

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Abstract

A sensitive reduction peak of verapamil is obtained by adsorptive stripping voltammetry in 0.01 M phosphate (pH 7.4) at an accumulation time of 30 s. The peak potential is −1.81 V (vs. Ag/AgCl). The peak current is directly proportional to the concentration of verapamil (1×10^{-8}–1×10^{-6} M), with a 3σ detection limit of 5×10^{-10} M (0.246 ng/ml). The R.S.D. at the 1×10^{-7} M level is 1.8%. The interference of some metal ions, and some amino acids, and the application of the method to analysis of urine, and pharmaceutical formulations are described. The method is simple (no extraction), rapid (30 s accumulation time), sensitive (the detection limit of verapamil is 0.491 ng/ml), reproducible(within day R.S.D. of 1.28–1.8%), and suitable for routine analysis of verapamil, urine, and pharmaceutical formulation.
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1. Introduction

All antihypertensive agents act at one or more of the four anatomic control sites, and produce their effects by interfering with normal mechanisms blood pressure [1]. A useful classification of these agents categorizes them according to principal regulatory site or mechanism on which they act. The categories include the following:

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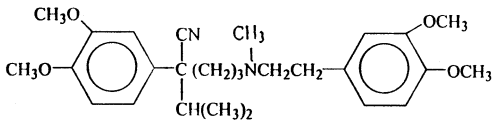
(i) Diuretics, which lower blood pressure by depleting the body of sodium and reducing blood volume.
(ii) Sympathoplegic agents, which lower blood pressure by reducing peripheral vascular resistance inhibiting cardiac function, and increasing venous pooling in capacitance vessels.
(iii) Direct vasodilators, which reduce pressure by relaxing vascular smooth muscle.
(iv) Agents that block production, which reduce peripheral vascular resistance and blood volume.

Verapamil is the first clinically useful member of the calcium channel-blocking drug [2]. It acts as an anti-anginal and antiarrhythmic [3]. Its generic name is verpamil hydrochloride and trade name is calan or Isoptin. The structural formula of verapamil is:



Various methods have been employed for the determination of verapamil; Issa et al. [4] have used the charge transfer complex formation to determine verapamil in isoptin tablet, spectrophotometric method has been used for determination of verapamil in sugar-coated tablet, and in biological fluids, tissues, and its dosage forms [5–7]. Several high-performance liquid chromatographic (HPLC) methods have been published for measuring the concentration of verapamil, and its active metabolite, norverapamil, in plasma, in urine, in drugs, in postmortem, and clinical samples [8–22]. Gas chromatographic (GC) and gas-liquid chromatographic (GLC) determination of verapamil in plasma and urine were studied by several authors [23–25]. Determination of verapamil hydrochloride using potentiometric and conductometric methods was described by Nikolic and coworkers [26].

Polarographic methods are ideally suited for the analysis of urinary metabolites since they are usually very polar compounds. This is in contrast to gas chromatographic methods which often require derivatization to reduce the polarity of the compounds to yield volatile derivatives suitable for analysis [27].

So far, it seems, no report has appeared in the literature describing the analytical utility of stripping voltammetry in the determination of verapamil. The present study deals with the quantitative determination of verapamil in pure drug, pharmaceutical formulation and in biological sample using direct current stripping voltammetric method. Stripping voltammetry is an important technique for trace determination of many organic and inorganic substances [28]. The adsorptive stripping technique has been used successfully for the determination of subnanogram levels of several drugs [29–33]. This technique eliminates both time-consuming solvent extraction steps and calculations of recovery common to photometric and chromatographic methods, while the resulting accuracy and precision are at least comparable if not better than the above methods. This technique is simple, rapid, sensitive, reproducible and easy to apply in routine usage.

2. Experimental

2.1. Instrumentation

Stripping and cyclic voltammograms were obtained using an EG&G Princeton Applied Research Corporation model 264A polarographic analyzer/stripping voltammeter, coupled with a PAR Model 303A, with a three-electrode system consisting of a hanging mercury drop electrode (HMDE) as working electrode, an Ag/AgCl (sat. KCl) reference electrode and platinum counter-electrode. The electrolytic cell was 10 ml. A PAR 305 stirrer was connected to the PAR 303A SMDE. A PAR model RE 0089 X-Y recorder was used to collect the experimental data. The HPLC system used in this study is Knaur autosystem, pump K-1001, Marthon Basic-plus autosampler (spark Holland), UV-Detector K-2600 adjusted at 235 nm. Data acquisition software (controller) Eurochrom 2000, Ver 1.6 Kanuer Berlin.

2.2. Reagents

A 0.01 M stock standard solution of verapamil hydrochloride (Sigma Chemical Co., USA) was daily prepared in bidistilled water and stored in the dark at 8 °C. Solutions of 0.1 M Britton–Robinson universal buffer, borax, sodium acetate, sodium nitrate, sodium perchlorate, potassium chloride, sodium borate and sodium phosphate buffer were prepared and used as the supporting electrolytes. Solution of 1 mM copper(II), lead(II), zinc(II), cobalt(II), nickel(II), cadmium(II) nitrate and the amino acids (glycine, L-ascorbic and L-aspartic acid) were prepared and used in the interference studies. Urine samples were taken from healthy persons.

2.3. Procedure

A 10 ml volume of 0.01 M phosphate buffer containing a specific amount of sample solution was added to the cell and purged with purified nitrogen for about 16 min to remove oxygen. The preconcentration potential (−1.1 V) was applied to a new mercury drop for 30 s. The voltammogram was recorded by using linear sweep voltammetry at a scan rate of 100 mV s^{-1}. The scan was terminated at −2.0 V. All the experiments were obtained at room temperature 25±1 °C.

The selectivity of the method has been tested. All solutions which were used in the proposed method were purely available, no peak interferences have been noted.

2.4. Analysis of ampoule

The whole content of one ampoule (Isoptin) [5 mg/2 ml i.e. each 1 ml of solution contains 2.5 mg verapamil HCl and 8.5 mg sodium chloride in water for injection [34]] was diluted with doubly distilled water in 25 ml measuring flask, then analysis was done by the recommended procedure.

2.5. Analysis of urine

A urine sample (10 μl) taken from a healthy person was added to the polarographic cell containing the supporting electrolyte (0.01 M phosphate buffer pH 7.4) i.e. the dilution factor of the urine sample in the cell was 1:1000. The voltammogram was recorded, then 10 μl spikes of the standard solution of verapamil were introduced into the cell and the voltammogram was recorded after each addition.

3. Results and discussion

3.1. Selection of experimental conditions

Various supporting electrolytes such as Britton–Robinson buffer, phosphate buffer, sodium acetate, sodium nitrate, sodium perchlorate, sodium citrate, sodium borate and potassium chloride were tested by adsorptive stripping voltammetry, and phosphate buffer was found to be the best, the voltammogram of verapamil being well defined and the sensitivity reasonably high. The effect of the concentration of phosphate buffer as the supporting electrolyte (0.01, 02, 0.05, and 0.1 M) and also the influence of pH (5.2–11.2) were studied. The highest peak current was obtained at 0.01 M phosphate buffer at pH 7.4. pH values of the buffer (7.25, 7.3, 7.4, 7.45) were tested in the presence of 1×10^{-6} M verapamil, it is found that the mean peak current is 4.7 μA with relative standard deviation of 1.38%.The effect of the preconcentration potential on the peak current was studied. The experiments showed that the peak current (Ip_c) increased as the potential changed from −0.6 to −1.1 V, and decreased when the preconcentration potential was more negative than −1.1 V. Therefore, a potential of −1.1 V was used as the accumulation potential for all experimental measurements.

3.2. Adsorptive properties

3.2.1. Cyclic voltammetry

When the cyclic voltammogram was taken for 1 μM verapamil in the presence of 0.01 M phosphate buffer (pH 6.9) at 30 s preconcentration time, a cathodic reduction peak is observed at −1.84 V in the first cycle. Subsequent scans (cycles 2, 3, 4, ...) exhibited a substantial increase in the peak to constant value, and no anodic peak was observed

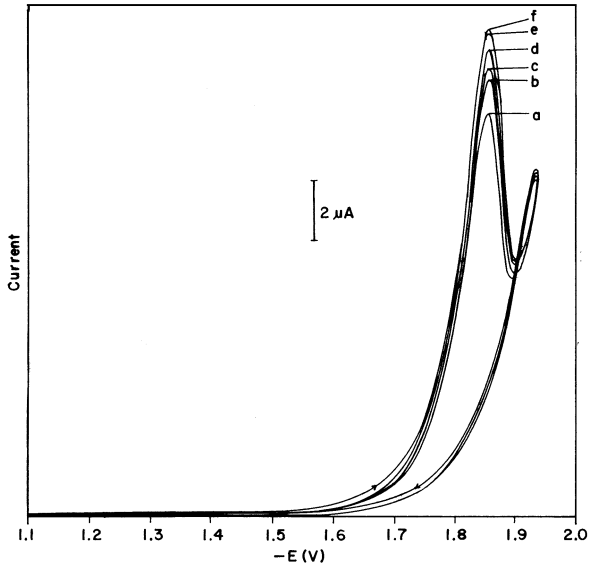


Fig. 1. Repetitive cyclic voltammograms for 1×10^{-6} M verapamil in 0.01 M phosphate buffer (pH 6.9) at accumulation potential −1.1 V, accumulation time 30 s and scan rate 100 mV/s. (a) First cycle, (b) second cycle, (c) third cycle, (d) fourth cycle, (e) fifth cycle, (f) sixth cycle.

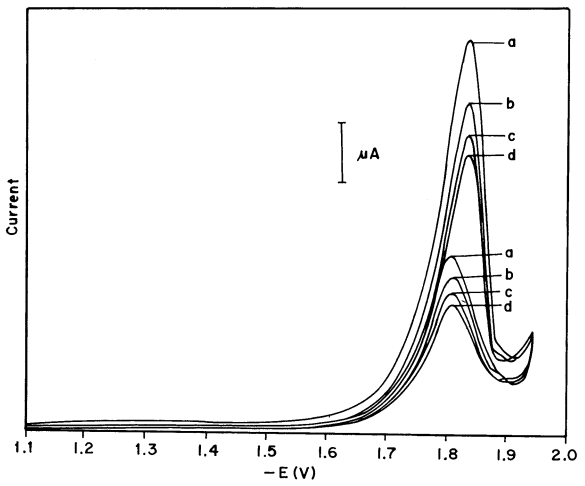


Fig. 2. Repetitive cyclic voltammograms for 1×10^{-6} M verapamil in 0.01 M phosphate buffer (pH 7.4) at accumulation potential −1.1 V, accumulation time 30 s and scan rate 100 mV/s. (a) First cycle, (b) second cycle, (c) third cycle, (d) fourth cycle.

on scanning in the negative direction as shown in Fig. 1, indicating that the electrode process is irreversible. At pH 7.4 a cathodic peak is observed at about −1.83 V. The repetitive cyclic voltammograms show that the peak current decreases sharply in the second and third cycles (Fig. 2), indicating the rapid desorption of the analyte. In the positive direction, a cathodic branch −1.83 V is formed maybe as a consequence of the reduction of the –C≡N to HC=NH at higher potential; the reduction of HC=NH is formed in lower potential than –C≡N in the second direction (anodic direction) at −1.81 V, or maybe the reduction of the racemic mixture for verapamil.

3.3. Effect of accumulation time

The amount of verapamil on the electrode surface increased as the deposition time increased (Fig. 3) and also enhancement of the peak current was observed. Fig. 4 shows plots of cathodic peak current (Ip_c) for linear sweep voltammetry versus accumulation time (t_{acc}) for different concentration of verapamil. At first, Ip_c increased linearly with t_{acc}, indicating that, before adsorptive equili-

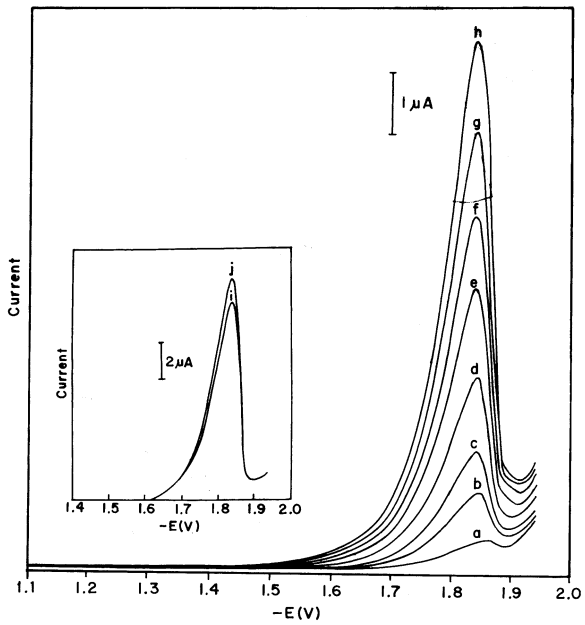


Fig. 3. Typical linear sweep cathodic stripping voltammograms for 3×10^{-7} M verapamil in 0.01 M phosphate buffer (pH 7.4) at accumulation potential −1.1 V, scan rate of 100 mV/s, and different accumulation times. (a) 0, (b) 15, (c) 30, (d) 60, (e) 90, (f) 120, (g) 150, (h) 180, (i) 240, (j) 300 s.

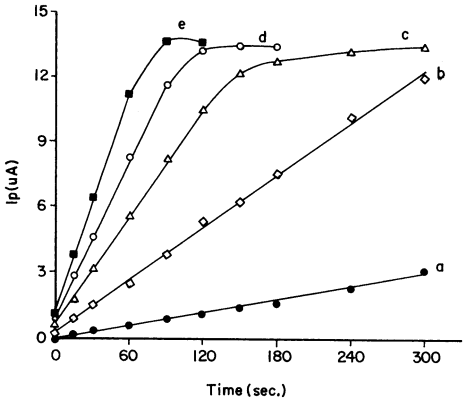


Fig. 4. Effect of preconcentration time on the cathodic stripping voltammograms of verapamil in 0.01 M phosphate buffer (pH 7.4) at accumulation potential −1.1 V, scan rate of 100 mV/s, and different concentrations: (a) 1×10^{-7}, (b) 3×10^{-7}, (c) 5×10^{-7}, (d) 7×10^{-7}, and (e) 9×10^{-7} M verapamil.

brium is reached i.e. surface coverage, the longer the preconcentration time, the more drug was adsorbed, and the larger the peak current. However, after a specific accumulation time (at concentration of verapamil up to 3×10^{-7} M), the peak current tended to level off, illustrating that adsorptive equilibrium of verapamil on the mercury electrode surface was achieved [35]. The collected data are illustrated in Table 1.

3.4. Effect of scan rate

The effect of scan rate on the peak current and peak potential was studied. The relationship between Ip_c or Ep_c and v at 30 s accumulation time shows a straight line with slope of 0.61 and correlation coefficient 0.9975 ($n = 5$). A slope of 1.0 is expected for an ideal reaction of surface species [36]; the peak potential was shifted to more negative value with increasing the scan rate.

3.5. Effect of concentration of verapamil

The effect of the concentration of verapamil at different accumulation times (60, 120, 180 s) on the peak current was studied. Calibration graphs for the analyte were linear from 1×10^{-8} up to 1×10^{-7} M at 180 s accumulation time with a correlation coefficient of 0.9995. But the linearity was observed from 1×10^{-7} M to 1×10^{-6} M at 60 s and from 1×10^{-7} M to 6×10^{-7} M at 120 s accumulation time, with correlation coefficient of 0.9992 and 0.9994, respectively, then a deviation from the linearity was observed at concentrations higher than 6×10^{-6} M. The mean calibration curves obtained were described by the following equation $Y = B_x + A$ ($n = 5$). The precision (expressed as % relative standard deviation R.S.D.) of the slope and intercept of the calibration lines were ranged 2.8–4.6% and 2.4–4.3%, respectively.

The collected from the regression analysis are illustrate in Table 2. The linear regression mode is the best one to fit the collected results for the concentration of 1×10^{-8} up to 1×10^{-7} M with 180 s and 1×10^{-7} up to 1×10^{-6} M at 30 and 60 s. Where the power regression mode is the most suitable one for the results of 1×10^{-7} to 1×10^{-6} M at 15 s and 1×10^{-7} to 6×10^{-7} M at 120 s.

3.6. Reproducibility

The high sensitivity of adsorptive cathodic stripping voltammetry is accompanied by the good reproducibility of the results. Five successive measurements of 1×10^{-7} M verapamil at pre-

Table 1
Characteristics of current–time curves established using different verapamil concentrations in 0.01 M phosphate buffer (pH 7.4)

Verapamil (M)	Equation[a]	Linearity range(s)	RSD for slope	RSD for intercept	Correlation coefficient
1×10^{-7}	$Y = 0.0099x \times -0.0221$	0–300	0.15	0.22	0.9962
3×10^{-7}	$Y = 0.0390x \times +0.3538$	0–300	0.22	0.18	0.9990
5×10^{-7}	$Y = 0.0782x \times +0.8540$	0–150	0.17	0.23	0.9985
7×10^{-7}	$Y = 0.1023x \times +1.6366$	0–90	0.25	0.15	0.9996
9×10^{-7}	$Y = 0.1322x \times +2.3051$	0–60	0.19	0.24	0.9998

[a] Y, the peak height in μA; x, the time in s.

Table 2
Characteristics of current–concentration curves established using different preconcentration times (15–180 s) for verapamil in 0.01 M phosphate buffer (pH 7.4)[a]

Regression mode	Accum. time (s)	Concentration linearity (M)	Slope (μA) 10^{-8} or 10^{-7} M	Intercept (μA)	Standard deviation	Correlation coefficient
Linear	180	$(1–10)\times10^{-8}$	0.1560	0.0120	0.3776	0.9995
	15	$(1–10)\times10^{-7}$	0.4625	−0.4325	0.4318	0.9979
	30	$(1–10)\times10^{-7}$	0.7490	−0.4910	1.3230	0.9987
	60	$(1–10)\times10^{-7}$	0.9152	−0.4533	0.8672	0.9992
	120	$(1–10)\times10^{-7}$	1.7486	−0.8867	0.7643	0.9964
Power	180	$(1–10)\times10^{-8}$	0.8971	−0.7172	0.0812	0.9994
	15	$(1–10)\times10^{-7}$	1.4819	−0.8044	0.09543	0.9990
	30	$(1–10)\times10^{-7}$	1.3172	−0.4331	0.1574	0.9984
	60	$(1–10)\times10^{-7}$	1.1987	−0.2386	0.2132	0.9988
	120	$(1–10)\times10^{-7}$	1.1914	0.0558	0.2413	0.9979
Exponential	180	$(1–10)\times10^{-8}$	0.2089	−0.6276	2.436	0.9611
	15	$(1–10)\times10^{-7}$	0.3828	−0.7537	1.546	0.9386
	30	$(1–10)\times10^{-7}$	0.3398	−0.3871	2.147	0.9368
	60	$(1–10)\times10^{-7}$	0.2725	−0.1034	3.165	0.9380
	120	$(1–10)\times10^{-7}$	0.4128	−4.2330	2.753	0.9761
Logarithmic	180	$(1–10)\times10^{-8}$	0.5896	−0.0156	3.2130	0.9517
	15	$(1–10)\times10^{-7}$	1.5704	−0.2718	3.7521	0.9160
	30	$(1–10)\times10^{-7}$	2.5661	−0.2622	4.1203	0.9407
	60	$(1–10)\times10^{-7}$	3.5864	−0.8370	3.6240	0.9481
	120	$(1–10)\times10^{-7}$	4.6978	0.0820	3.8761	0.9482

[a] Y, the peak height in μA; x, the concentration in molar.

concentration time 60 s were studied. The relative standard deviation was 1.8%.

The experiment of the above solution was examined by making three consecutive analyses after 10 h; no change is found in the response of the peak current with R.S.D. 0.8% ($n=5$). This means that the drug is stable in the solution and during the actual analysis.

3.7. Detection limit

The general definition given in the literature ($y\beta+3\sigma\beta$) was used for the estimation of the limit of detection (L.O.D.) [37,38]. The calculated L.O.D. of verapamil in aqueous solution using preconcentration time 300 s is 1×10^{-9} M, which is equal to 0.491 ng/ml of verapamil.

Limit of quantitation was also estimated using the following equation [39] LOQ $=10\sigma/S$, which is found to be 30 ng/ml for the ranges 4.91–49.11 ng/ml and 49.11–491.1 ng/ml verapamil. The relative standard deviation was 1.6% (five replicates) with of 0.9968 correlation coefficient. The accuracy of the method was investigated by determining the recovery (99.86%) of a definite concentration 0.491 ng/ml (1×10^{-9} M) verapamil ($n=5$).

4. Effect of interferences

On the addition of 1×10^{-7} to 1×10^{-5} M of Cu(II), Ni(II), Co(II), Pb(II), Cd(II) and Zn(II) ions individually or admixture to 1×10^{-7} M verapamil, no change in the peak current of the drug was observed. Also the addition of the amino acids such as glycine, L-ascorbic, and L-aspartic acids (1×10^{-7}–1×10^{-5} M) to verapamil solution exhibits no change in the peak current of verapamil.

5. Analytical applications

5.1. Determination of verapamil in pharmaceutical formulation

The whole content of one ampoule (Isoptin) 5 mg/2 ml verapamil was diluted with doubly distilled water in 25 ml measuring flask. 10 μl was introduced in the polarographic cell containing 0.01 M phosphate buffer, and the content of the ampoule was determined by the standard addition method. On plotting of peak height versus concentration of verapamil, a straight line is obtained over the range of 1×10^{-7} to 6×10^{-7} M. The average percentage recovery was 98.3% ($n=5$) with 1.3% RSD and 0.9992 correlation coefficient.

5.2. Determination of verapamil in biological samples

The measurement of verapamil in urine sample was demonstrated as follows. The sample was diluted (1:1000) with supporting electrolyte (0.01 M phosphate buffer) and increasing verapamil concentration. The standard addition voltammograms are shown in Fig. 5. The resulting peak currents show a linear behavior with the concen-

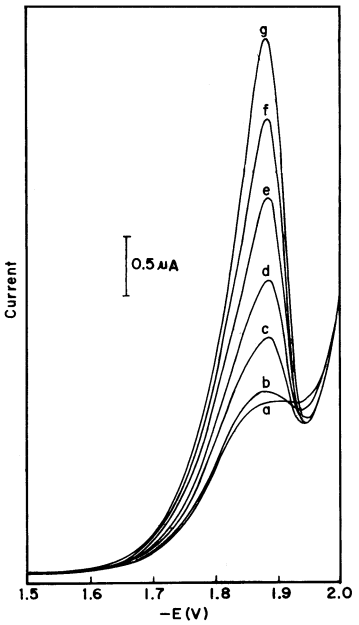


Fig. 5. Typical linear sweep cathodic stripping voltammograms of urine sample (1:1000) 0.01 M phosphate buffer (pH 7.4) at accumulation potential −1.1 V, accumulation times 60s and scan rate of 100 mV/s. (a) Urine sample, (b) at 1×10^{-7}, (c) at 2×10^{-7}, (d) at 3×10^{-7}, (e) at 4×10^{-7}, (f) at 5×10^{-7}, (g) at 6×10^{-7} M verapamil.

tration over the range of 1×10^{-7} to 6×10^{-7} M verapamil ($r = 0.9978$). Therefore 49.11 mg/l verapamil in the original sample can be determined using accumulation time 60 s. The recovery was found to be 99.8%. The repeatability and reproducibility of the results were tested and the relative standard deviation were found to be 1.5% ($n = 5$).

Stability of urine samples kept in an ice bath was tested by making five consecutive analyses of the same sample over a period of approximately 6 h. There were no significant changes in the peak currents between the first and the last samples.

The present method of verapamil determination was compared with that of Harapat and Kates [40] using high pressure liquid chromatography, where good agreement in the results has been observed i.e. in the same order of accuracy and precision.

Robustness is the capacity to remain unaffected by small, but deliberate, variations in method parameters [41].The robustness of this method (linear sweep stripping voltammetry (LSSV)) was examined by varying the pH, the concentration of supporting electrolyte, temperature, and stability of sample solution. No significant change in any of the observed parameters was found.

6. Conclusion

The stripping voltammetric method is found to be practically rapid, convenient, accurate, and precise in the analysis formulation of drug and biological fluids. As low as 0.491 ng/ml of verapamil was accurately measured by the proposed method. The method can be used successfully to assay the drug in dosage form as well as in spiked urine.

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