

Direct Detection of Drugs of Abuse in Whole Hemolyzed Blood Using the EMIT d.a.u. Urine Assays*

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Abstract

A simple, rapid, and sensitive method for the direct detection of a broad spectrum of drugs of abuse in hemolyzed whole blood by the enzyme multiplied immunoassay technique (EMIT) is described. A methanolic extract of 1 mL of whole blood is directly analyzed with EMIT urine assays. The proposed method is very sensitive and can detect drug concentrations in low therapeutic to subtherapeutic concentration ranges for all ten assays used. The EMIT urine assays used in this study included those for opiates, amphetamines, methadone, barbiturates, phencyclidine (PCP), methaqualone, propoxyphene, cocaine metabolite, benzodiazepine metabolite, and cannabinoids. This method should be most useful for screening forensic cases by EMIT when no urine is available, such as in impaired driving cases.

Introduction

In 1972 Rubenstein et al. (1) reported the first use of an enzyme multiplied immunoassay technique (EMIT) for the screening of urine for drugs of abuse. Since that time, enzyme immunoassay techniques have seen ever increasing usage. Currently available EMIT assays for the detection of drugs of abuse in urine include those for amphetamines, barbiturates, cocaine metabolite (benzoylecgonine), benzodiazepine metabolite, propoxyphene, methaqualone, phencyclidine, opiates, methadone, and cannabinoids. These urine assays have only a limited use in forensic toxicology. In an attempt to broaden the usefulness of EMIT, saliva (2,3), vitreous humor (4), and tears (5,6) have been analyzed directly in lieu of urine. It has not been possible to directly analyze whole blood, bile, or tissue extracts by EMIT due to high background absorbance levels (7), but the EMIT procedures have been successfully modified for use with blood, bile, and tissue extracts (8,9). These procedures involve relatively large volumes (5-10 mL) of blood, lengthy extraction procedures with water immiscible organic solvents, evaporation to dryness, and reconstitution with buffer. Several different extraction procedures are required to do a comprehensive drug screen.

Until recently, most of the Syva EMIT d.a.u.[®] (drugs of abuse in urine) assays used a lysozyme-mediated reaction. In 1981, Fletcher et al. (10) noted that this enzyme reaction could be performed in an environment containing as much as 75 percent methanol. Consequently, a methanolic extract of whole blood could be analyzed directly by radioimmunoassay (RIA) for the presence of tetrahydrocannabinol (THC). Peel and Perrigo (12) have used an assay mediated by malate dehydrogenase to detect cannabinoids in a methanolic extract of whole blood. In early 1985, Syva converted seven EMIT d.a.u. assays from the lysozyme-mediated reaction to one utilizing glucose-6-phosphate dehydrogenase (13). Currently, all ten EMIT urine assays utilize glucose-6-phosphate dehydrogenase. This conversion has allowed increased sensitivity with fewer interferences.

The purpose of this investigation was first to determine whether the methanolic extraction procedure used by Peel and Perrigo (12) can be used with the recently available glucose-6-phosphate dehydrogenase mediated EMIT assays. The ten currently available EMIT assays were found to function well in a methanolic environment. The next step was to develop a simple, rapid method allowing all ten EMIT urine assays to be used for the direct analysis of a methanolic extract of 1 mL of blood. The final purpose of this investigation was to determine the minimum detectable concentration limit with a confidence of three standard deviations for each drug assay. EMIT assays have traditionally been criticized for not being as sensitive as RIA (7,14). It was our intention to determine whether a methanolic extract of 1 mL of blood can be assayed by all ten EMIT urine assays with sufficient sensitivity to enable the detection of drugs of abuse at low or subtherapeutic concentrations.

Materials

Instruments

A Syva EMIT system was used. It consisted of a Stasar-III Spectrophotometer (Gilford Instrument Labs), a Syva pipetter-diluter (Cavro Scientific Instruments), an EMIT Clinical Processor Model CP-5000, and a Gilford 3021 Vacuum Receiver and DeVilbiss pump. The spectrophotometer was operated in the absorption mode at 340 nm, and the microflow cell was set at 30°C. The pipetter-diluter was set to aspirate 50 μ L of sample and to deliver the sample plus 250 μ L of buffer. The CP-5000

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was timed to measure absorbances at 15 and 95 s and to calculate the absorbance difference (ΔA).

The methanolic supernatant was filtered with a 0.45- μm disposable filter assembly (Gelman Acrodisc, Product No. 4184) and a 1-cm³ tuberculin disposable syringe (Becton Dickinson & Co. Ltd.).

Reagents

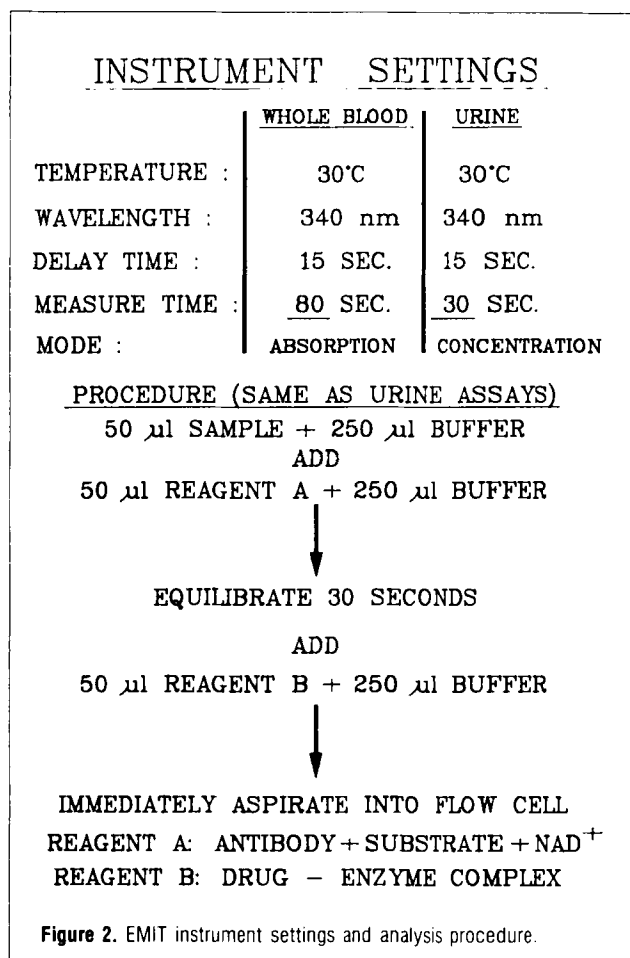
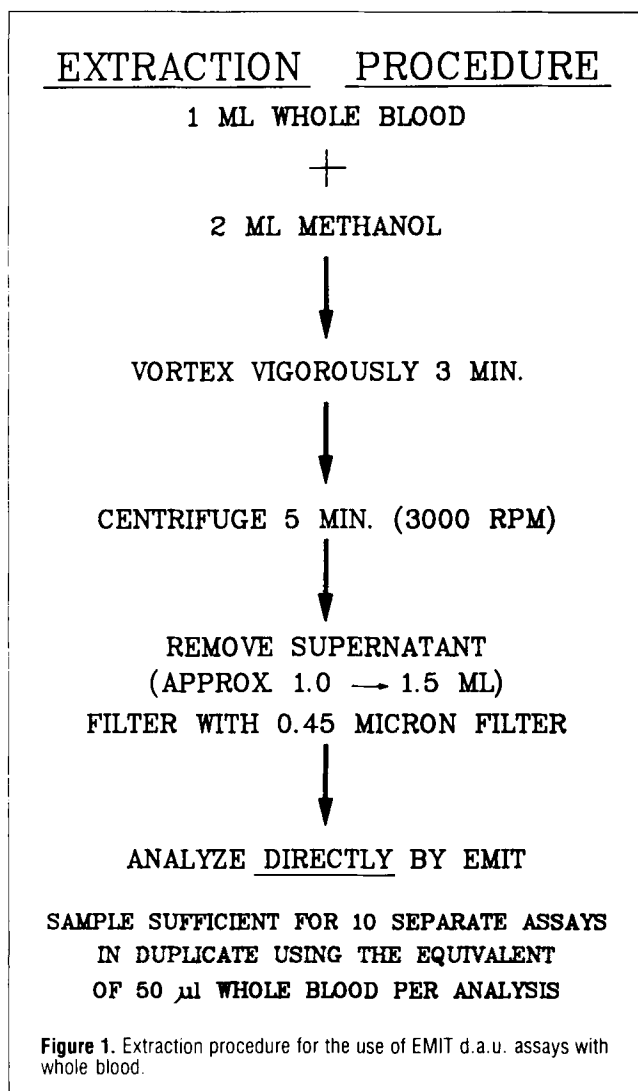
The following ten EMIT d.a.u. assay kits were purchased from the Syva Company and prepared as directed: amphetamine, barbiturate, benzodiazepine metabolite, cannabinoid, cocaine metabolite, methadone, methaqualone, opiate, phenacyclidine, and propoxyphene. Reagent A for each assay contained antibody, substrate (glucose-6-phosphate), and coenzyme (nicotinamide adenine dinucleotide (NAD⁺)) in 0.055M Tris-HCl, pH 5.2. Reagent B contained a drug derivative labelled with glucose-6-phosphate dehydrogenase and 0.05% sodium azide in 0.055M Tris-HCl, pH 8.0. Buffer concentrate contained 0.825M Tris-HCl buffer, pH 8.0 and 0.05% sodium azide. Methanol was purchased from Caledon and was HPLC grade.

The following drugs or metabolites (obtained from the Department of Health and Welfare, Ottawa) were prepared in ethanol at a concentration of approximately 1 mg/mL calculated as the free base or acid: dextroamphetamine, secobarbital,

oxazepam, tetrahydrocannabinol (THC), benzoylecgonine, methadone, methaqualone, morphine, phenacyclidine, and propoxyphene. Each stock solution was diluted 1:10 with water. Blood (10 mL) was spiked quantitatively with each aqueous stock solution over a concentration range from subtherapeutic to toxic, including blanks. One set of blood drug standard solutions contained the following drugs: dextroamphetamine, benzoylecgonine, methadone, morphine, and secobarbital (corresponding to the Syva A calibrator solutions). Another set of blood drug standards contained methaqualone, oxazepam, phenacyclidine, and propoxyphene (corresponding to the Syva B calibrator solutions). The cannabinoid blood standards were prepared from an ethanolic stock solution of freshly prepared tetrahydrocannabinol at a concentration of 1 mg/mL. The blood, which had been frozen, was bovine blood containing 1% sodium fluoride and 0.25% potassium oxalate. All reagents and standards, refrigerated for storage, were allowed to equilibrate for at least 2 hours at room temperature before use.

Procedure

One milliliter of blood was added to a 13-mL plastic centrifuge tube and 2 mL of methanol was added by streaming down the inside of the tube. The mixture was vigorously vortexed for 3-5 min and then centrifuged at 3000 rpm for 5 min at -20°C. The clear supernatant (1.0-1.5 mL) was filtered with a 0.45- μm filter attached to a 1-cm³ tuberculin syringe. As much sample as possible was removed from the filter assembly by purging



with air. After equilibration to room temperature, the filtered supernatant was analyzed directly by EMIT. This procedure is outlined in Figure 1. With the pipetter-diluter, 50 μL of methanolic supernatant was added to 250 μL of buffer solution and mixed in a 2-mL disposable beaker. Fifty microliters of reagent A (antibody, substrate, and coenzyme) and 250 μL of buffer were added to the beaker. After a 30-s equilibration, 50 μL of reagent B (drug-enzyme complex) and 250 μL of buffer were added to the beaker. The contents of the beaker were immediately aspirated into the flow cell of the spectrophotometer, and absorbance readings were taken automatically at 15 and 95 s. This procedure is outlined in Figure 2.

Results and Discussion

The standard curves for each of the ten assays are shown in Figures 3 to 12. Each sample was analyzed in duplicate, and

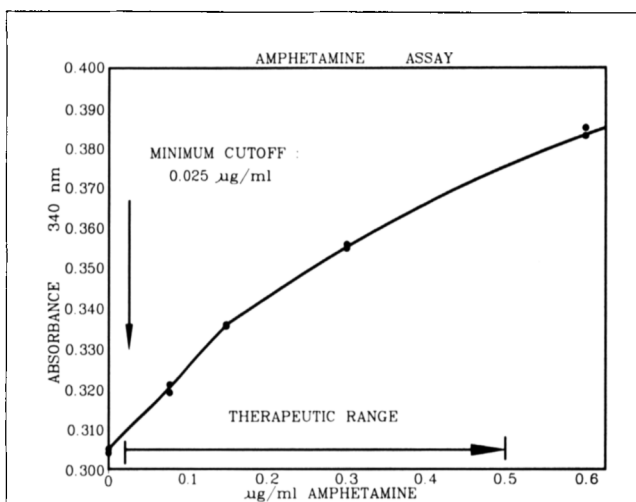


Figure 3. Standard curve of the EMIT d.a.u. amphetamine assay using a methanolic extract of whole blood. Concentration of amphetamine ($\mu\text{g}/\text{mL}$) vs. the change in absorbance at 340 nm.

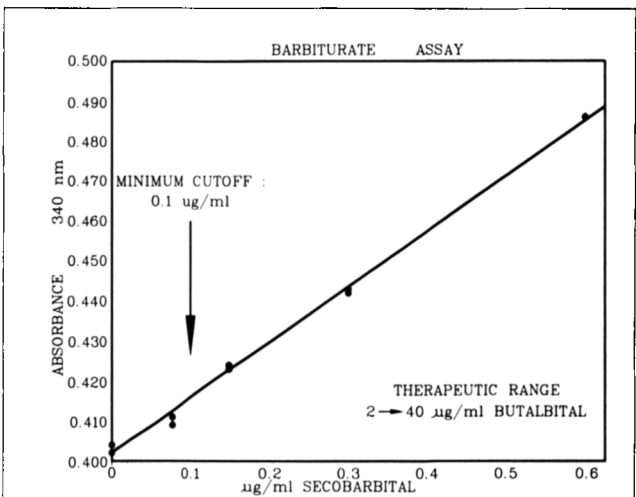


Figure 4. Standard curve of the EMIT d.a.u. barbiturate assay using a methanolic extract of whole blood. Concentration of secobarbital ($\mu\text{g}/\text{mL}$) vs. the change in absorbance at 340 nm.

blanks accompany each assay. The therapeutic drug concentration range is indicated in each figure. For the barbiturate assay, the therapeutic range of butalbital is shown, rather than that of secobarbital, as it is one of the most potent barbiturates. For the cocaine metabolite assay, the therapeutic range of cocaine is shown. The cannabinoid assay standard curve was prepared with tetrahydrocannabinol (THC), because the carboxylic acid metabolite (THC-COOH) was not available. The therapeutic range shown in Figure 6 corresponds to the estimated

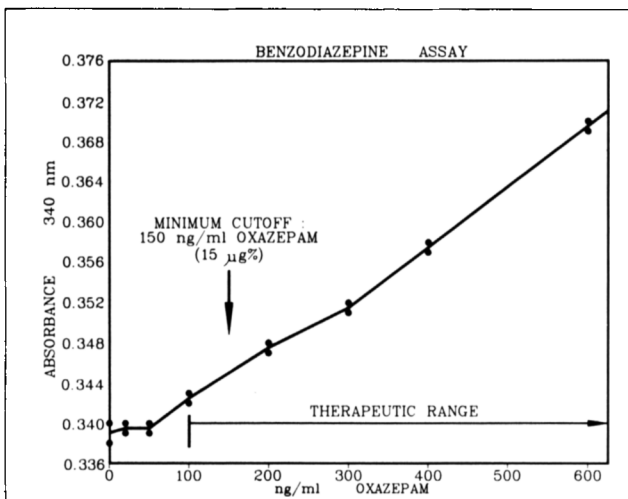


Figure 5. Standard curve of the EMIT d.a.u. benzodiazepine metabolite assay using a methanolic extract of whole blood. Concentration of oxazepam ($\mu\text{g}/\text{mL}$) vs. the change in absorbance at 340 nm.

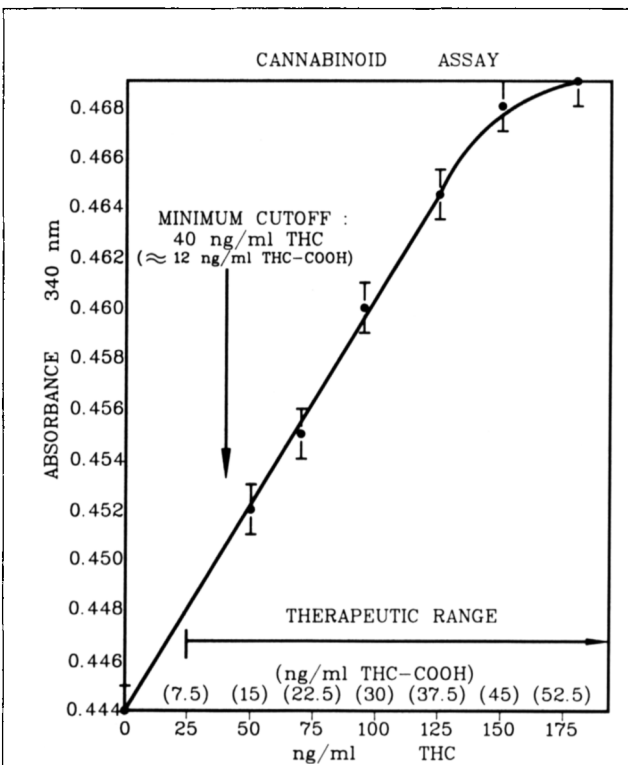


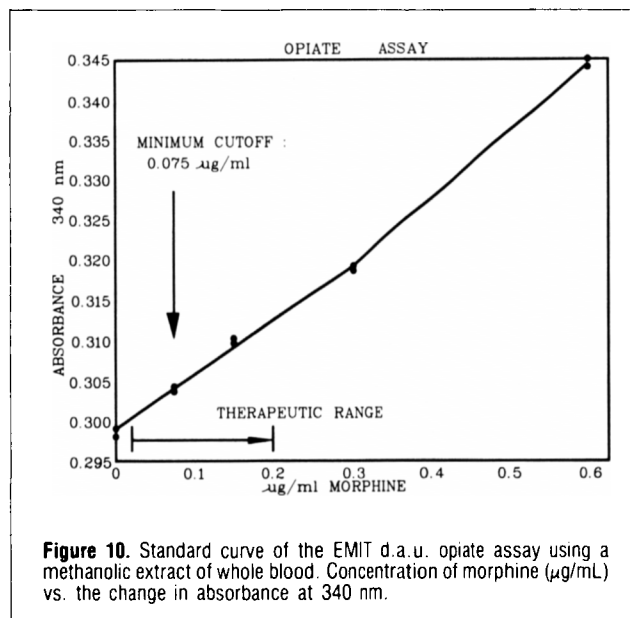
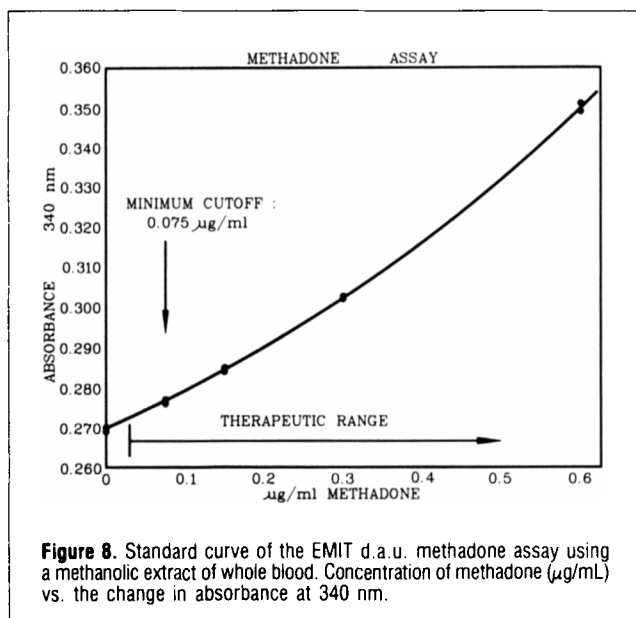
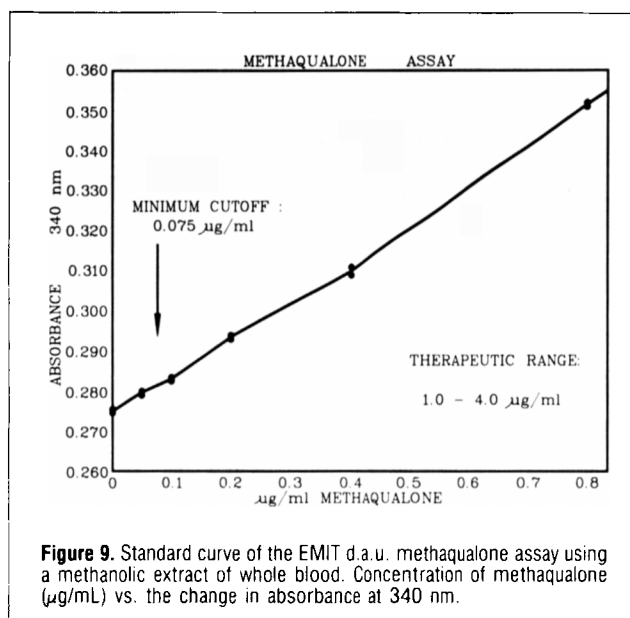
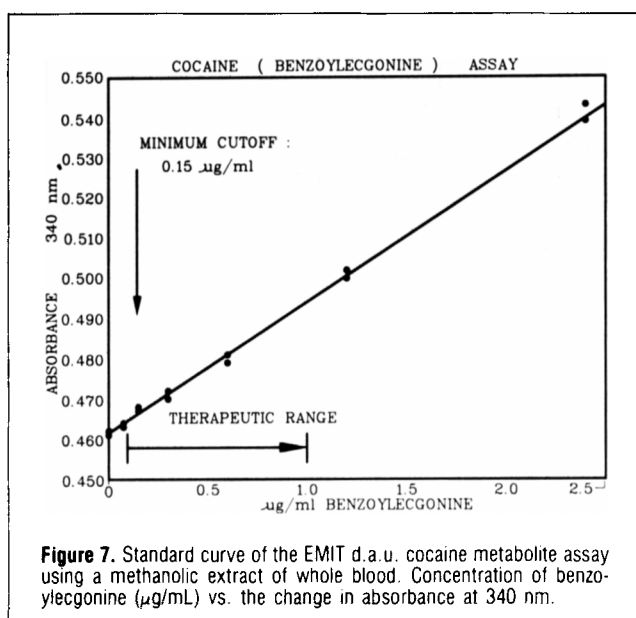
Figure 6. Standard curve of the EMIT d.a.u. cannabinoid assay using a methanolic extract of whole blood. Concentration of THC (ng/mL) vs. the change in absorbance at 340 nm.

range shown for THC-COOH. Syva reports that the response of THC is less than that of THC-COOH in the cannabinoid assay (15). It is estimated that 20 ng/mL of THC-COOH will give the same response as 70 ng/mL of THC. In this procedure the minimum detectable blood concentration of THC would be approximately 40 ng/mL THC. This is a relatively high concentration of THC, but in terms of relative enzyme response it corresponds to approximately 12 ng/mL THC-COOH, to which the antibody is most sensitive. The metabolite THC-COOH reportedly appears in blood at higher concentrations for a number of hours after smoking marijuana, whereas THC disappears relatively quickly (16).

Fletcher (10) observed that the lysozyme-mediated assays could tolerate up to 75 percent methanol. Peel and Perrigo (12) showed that the cannabinoid assay using malate dehydrogenase was also tolerant of elevated methanol concentrations. It was obvious from the standard curves shown in Figures 3-12 that

the glucose-6-phosphate dehydrogenase mediated reaction was also tolerant of methanol at a concentration of approximately 66 percent. It should be noted that the final concentration of methanol in the sample cuvette would be approximately 3.7% by volume. As might be expected, the rate of reaction has been decreased, as noted by the change in absorbance per minute.

For urine analysis, Syva recommends a maximum allowable difference between replicates of 6 milliabsorbance (mA) units in the concentration mode, which incorporates an amplification factor of 2.667 relative to absorbance data. Thus the maximum allowable difference in the absorbance mode would be 6 divided by 2.667, or 2.25 mA units. Presumably this corresponds to 1 standard deviation. The recommended difference between the negative and low calibrators of 18 mA units in the concentration mode would correspond to 6.75 mA units in the absorbance mode. This value presumably corresponds to 3 standard deviations.



The proposed method using methanolic extracts of whole blood is performed in the absorbance mode rather than the concentration mode. Our results have shown that replicate analyses of methanolic extracts can achieve a difference of 2 mA units. This is best illustrated in Table I, where 20 samples of outdated whole blood obtained from the Red Cross (presumably drug-free) showed a standard deviation in nine assays as a mean value of 1.63 mA units. Three standard deviations would be 4.9 mA units. This data corresponds very well to the recommended values of 2.25 mA units and 6.75 mA units for urine analysis.

The minimum detection limit for each assay was determined to be the highest absorbance value for the negative calibrator plus 6 mA units (3 standard deviations). The minimum detectable drug concentration for each assay is shown in Table II. All of the levels shown correspond to low or subtherapeutic drug concentrations. These levels correspond to absolute minimum detection limits and may be somewhat low for routine toxicological drug screening. For routine work, the authors have adopted a cutoff of 10 mA units (approximately 5 standard deviations), rather than 6 mA units.

The data reported above was obtained with the flow cell temperature set at 30°C and a measure time of 80 s. Preliminary studies were conducted in the hope of lowering the minimum detection limits. One approach was to increase the temperature to 37°C. It was found that the rate of absorbance change did increase with the increase in temperature, and the absolute difference in absorbance was increased by an increase in measure time longer than 80 s. However, the relative change in absorbance did not change significantly. Therefore, in order to maintain the same level of precision and for practical considerations, the reaction conditions were not altered.

By using 1 mL of blood and 2 mL of methanol, the supernatant volume was between 1 and 2 mL, depending on the hematocrit. The methanolic extract was then analyzed by ten different assays. Each assay required 50 μ L of extract—100 μ L if done in duplicate. Since the maximum allowable difference between replicates was only 2 mA units, it was occasionally necessary to sample in triplicate. If it was necessary to sample each of the ten assays in triplicate, the volume of methanolic extract

required would be 1.5 mL (10 assays \times 3 samples \times 50 μ L per sample). The supernatant volume may, however, be only approximately 1 mL. Fortunately it has been the authors' experience that if a sample yields an absorbance value less than or equal to that of the negative calibrator, then only one analysis is required. Since most of the ten assays for any given blood sample will be negative, there should be more than enough supernatant for all 10 assays.

Occasionally it was found that blood with a very high hematocrit, such as heart blood, could yield an extract that was still pale red, indicating that not all of the hemoglobin had been precipitated. Usually these samples could still be analyzed directly, even though the initial absorbance values were somewhat elevated. If the analyst wants to remove the remaining hemoglobin, two procedures can be attempted. The first is to freeze the extract causing additional precipitation which can then be filtered off. Alternatively, additional methanol (0.5 mL) can be added, the sample revortexed and centrifuged. This additional procedure will result in a minor dilution relative to the standards. A number of actual forensic case blood samples and selected spiked blood samples were analyzed by the proposed method.

Five postmortem blood samples were analyzed by the amphetamine assay. The data is summarized in Table III. Case 4 was negative, while cases 1 and 2 showed a very weak positive response. Cases 3 and 5, which were very putrid, showed a strong positive response. The EMIT amphetamine assay is known to cross-react with a broad spectrum of phenethylamines, many of which are putrefactants (7,16). All five actual cases examined had been previously analyzed by thin-layer chromatography (TLC), gas chromatography with nitrogen specific detection (GC/NPD), gas chromatography/mass spectrometry (GC/MS), and EMIT on urine samples where possible. All five cases were found to contain no detectable amphetamine related drugs. Thus, with postmortem samples a higher minimum cutoff is recommended for the amphetamine assay—perhaps corresponding to 0.20 or 0.30 μ g/mL amphetamine. Due to the high cross-reactivity of this assay to putrefactants, a number of false positive cases can be expected. Antemortem samples, with no

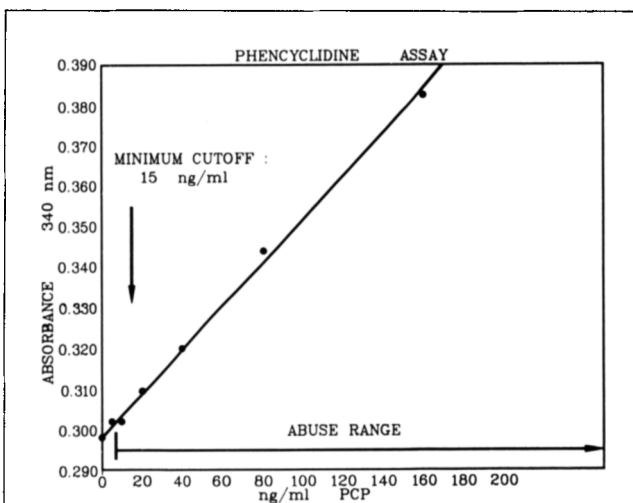


Figure 11. Standard curve of the EMIT d.a.u. phencyclidine assay using a methanolic extract of whole blood. Concentration of phencyclidine (ng/mL) vs. the change in absorbance at 340 nm.

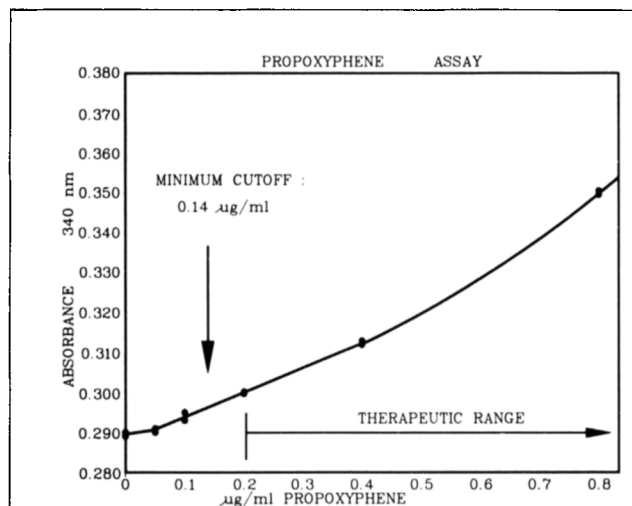


Figure 12. Standard curve of the EMIT d.a.u. propoxyphene assay using a methanolic extract of whole blood. Concentration of propoxyphene (μ g/mL) vs. the change in absorbance at 340 nm.

apparent putrefaction, should be used with the low calibrator cutoff of 0.025 $\mu\text{g}/\text{mL}$. A spiked blood sample containing a therapeutic concentration of methamphetamine (0.05 $\mu\text{g}/\text{mL}$) was analyzed and gave a response corresponding to 0.075 $\mu\text{g}/\text{mL}$ of apparent amphetamine. This indicated that the amphetamine assay was somewhat more sensitive to methamphetamine. It should be noted that in samples yielding a very strong positive response, the allowable maximum absorbance difference between replicate samples was not attained. At these high reaction rates the precision was observed to decrease somewhat.

Three actual cases known to be negative for barbiturates were analyzed by the barbiturate assay (Table IV). All three cases were negative as expected. The minimum detectable limit of secobarbital was 0.10 $\mu\text{g}/\text{mL}$, well below the therapeutic range of 2–10 $\mu\text{g}/\text{mL}$. Two samples of blood spiked with subtherapeutic concentrations of butalbital (0.50 and 1.0 $\mu\text{g}/\text{mL}$) were also analyzed. Our laboratory has found this potent barbiturate to be present in the blood of many impaired drivers. The 1.0- $\mu\text{g}/\text{mL}$ butalbital sample resulted in an apparent secobarbital concentration of 0.51 $\mu\text{g}/\text{mL}$, indicating that the barbiturate assay was approximately half as sensitive to butalbital as to secobarbital. Nevertheless, this assay could easily detect a subtherapeutic level of butalbital.

Four case bloods were analyzed by the benzodiazepine metabolite assay (Table V). Case 4, a known negative, was found to be negative by this assay. Case 1 was known to contain 160 ng/mL diazepam (as determined by gas chromatography with

electron capture detection, GC/ECD). This sample was negative, indicating that the assay was somewhat more sensitive to oxazepam than to diazepam. Case 2 was known to contain less than 10 ng/mL diazepam and 20 ng/mL nordiazepam (subtherapeutic concentrations). As expected, this sample gave a negative response. Case 3 was known to contain therapeutic levels of diazepam and nordiazepam (290 ng/mL and 800 ng/mL, respectively). This sample gave a strong positive response. A spiked blood sample containing 10 ng/mL triazolam, a potent benzodiazepine, was also analyzed. This therapeutic level of triazolam gave a negative response. Cases suspected of containing triazolam, alprazolam, or other new potent benzodiazepines could be screened by extracting a separate blood sample. A therapeutic level of triazolam (5 ng/mL) gave a positive result when 3 mL of blood was extracted with 5 mL of *n*-butyl chloride, the organic extract was evaporated to dryness, and the extract reconstituted in 200 μL of methanol/water (2:1).

Two case blood samples were analyzed by the cocaine metabolite assay (Table VI). Case 1 was known to be negative and was, in fact, below the minimum cutoff of 0.15 $\mu\text{g}/\text{mL}$ benzoylecgonine. Case 2 was from an overdose, and the cocaine blood concentration was known to be 4.7 $\mu\text{g}/\text{mL}$, determined by GC/NPD. The apparent benzoylecgonine concentration by the EMIT assay was 5.8 $\mu\text{g}/\text{mL}$. It should be noted that cocaine, benzoylecgonine, and other metabolites will react with the antibody and that the antibody is reported to be approximately 75 times less sensitive to cocaine than to benzoylecgonine (18).

Table I. Twenty drug-free human blood samples each assayed by nine separate EMIT d.a.u. assays. Mean $\Delta A_{340 \text{ nm}}$ values with standard deviation calculated.

ASSAY	n	MEAN $\Delta A_{340 \text{ nm}}$ ($\times 1000$)	1 STANDARD DEV. (σ)	3 σ
AMPHETAMINE	20	294.2	2.17	6.51
BARBITURATE	20	408.3	1.69	5.07
COCAINE	20	484.4	2.23	6.69
BENZODIAZEPINE	20	349.3	1.34	4.02
METHADONE	20	272.0	1.15	3.45
METHAQUALONE	20	208.4	1.76	5.28
OPIATE	20	297.4	1.87	5.61
PROPOXYPHENE	20	228.6	1.14	3.42
PHENCYCLIDINE	20	238.6	1.36	4.08
MEAN	20	309	1.63	4.90

Table II. Therapeutic concentration range and minimum detection limits (3 SD) for ten EMIT d.a.u. assays using whole blood.

ASSAY	THERAPEUTIC RANGE	MIN. DET. LIMIT (3 σ)
AMPHETAMINE	0.02 \rightarrow 0.5 $\mu\text{g}/\text{mL}$	0.025 $\mu\text{g}/\text{mL}$
BARBITURATES (BUTALBITAL)	2 \rightarrow 40 $\mu\text{g}/\text{mL}$	0.10 $\mu\text{g}/\text{mL}$
BENZODIAZEPINE (OXAZEPAM)	50 \rightarrow 500 ng/mL	150 ng/mL
CANNABINOID (THC-COOH)	25 \rightarrow 300 ng/mL (TOTAL CANNABINOID)	12 ng/mL (THC-COOH)
COCAINE METABOLITE (BENZOYLECGONINE)	0.10 \rightarrow 1.0 $\mu\text{g}/\text{mL}$	0.15 $\mu\text{g}/\text{mL}$
METHADONE	0.03 \rightarrow 0.5 $\mu\text{g}/\text{mL}$	0.075 $\mu\text{g}/\text{mL}$
METHAQUALONE	1.0 \rightarrow 10.0 $\mu\text{g}/\text{mL}$	0.075 $\mu\text{g}/\text{mL}$
OPIATES (MORPHINE)	0.02 \rightarrow 0.2 $\mu\text{g}/\text{mL}$	0.075 $\mu\text{g}/\text{mL}$
PHENCYCLIDINE	10 \rightarrow 250 ng/mL	15 ng/mL
PROPOXYPHENE	0.2 \rightarrow 2.0 $\mu\text{g}/\text{mL}$	0.15 $\mu\text{g}/\text{mL}$

Three case blood samples known to be negative for methaqualone were analyzed (Table VII). As expected, all three samples gave negative responses.

Three case blood samples were analyzed by the opiate assay (Table VIII). Case 1 was known to be negative and yielded a negative response by EMIT. Case 2 gave a strong positive response by the EMIT urine analysis. Free morphine was quantified in the blood by GC/ECD at 0.009 $\mu\text{g}/\text{mL}$. This sample yielded an apparent morphine level of 0.31 $\mu\text{g}/\text{mL}$ by the EMIT blood analysis. This discrepancy is undoubtedly due to the fact that morphine glucuronides were being detected by the EMIT but not by GC/ECD. Case 3 was a sample from a known heroin overdose. A free-morphine level of 1.1 $\mu\text{g}/\text{mL}$ was determined by GC/ECD. The EMIT blood analysis yielded an apparent morphine level greater than 2.5 $\mu\text{g}/\text{mL}$, an extremely high positive result.

Five case bloods known to be negative for phencyclidine (PCP) were analyzed (Table IX) and all gave a negative response.

Three case bloods were analyzed by the propoxyphene assay (Table X). Cases 1 and 2 were known to be negative, and this was confirmed by EMIT. Case 3 was known to contain 0.13 $\mu\text{g}/\text{mL}$ propoxyphene, determined by GC/NPD (a sub- to low therapeutic level). The apparent propoxyphene concentration as determined by EMIT was 0.14 $\mu\text{g}/\text{mL}$.

Summary

The data demonstrates the applicability of extending the EMIT d.a.u. assays to the analysis of whole hemolyzed blood. This can be done by directly analyzing a methanolic extract of whole blood. Advantages of this method include the following:

1. It extends the EMIT assay to more toxicologically significant specimens, namely whole hemolyzed blood, which is typically available in forensic cases.
2. The procedure is very simple and rapid.
3. Control of pH before extraction is not required.
4. Only one phase is produced with a protein precipitate, because methanol is miscible with water. As such, acidic, neutral, and basic drugs are all simultaneously extracted into the methanol-plasma supernatant.
5. A small sample size (1 mL) can be used for the duplicate analysis of 10 separate assays.
6. The minimum detectable concentrations correspond to low or subtherapeutic concentrations for the target drugs in each of the assays.
7. The method can be used for semiquantitation of drugs in whole blood. One must keep in mind, however, that immunoassay techniques can cross-react with drug analogues and metabolites.

Table III. EMIT d.a.u. amphetamine assay results for five case blood samples. Apparent EMIT results with GC results. Methamphetamine spiked blood sample data also included.

AMPHETAMINE ASSAY			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG.CAL	304 305		0 $\mu\text{g}/\text{ML}$
LOW CAL (MINIMUM)	310		0.025 $\mu\text{g}/\text{ML}$
MED CAL	320		0.075 $\mu\text{g}/\text{ML}$
CASE #1 (POST MORTEM)	314 315	APPARENT 0.05 $\mu\text{g}/\text{ML}$ AMPHETAMINE	NEGATIVE
CASE #2 (POST MORTEM)	310 310	APPARENT 0.025 $\mu\text{g}/\text{ML}$ AMPHETAMINE	NEGATIVE
CASE #3 (POST MORTEM)	499 510	APPARENT > 2.5 $\mu\text{g}/\text{ML}$ AMPHETAMINE (PUTRID)	NEGATIVE
CASE #4 (POST MORTEM)	304 306	NEGATIVE	NEGATIVE
CASE #5 (POST MORTEM)	457 466	APPARENT 2.1 $\mu\text{g}/\text{ML}$ AMPHETAMINE (PUTRID)	NEGATIVE
METHAM PHETAM INE STD	319 321	APPARENT 0.075 $\mu\text{g}/\text{ML}$ AMPHETAMINE	0.05 $\mu\text{g}/\text{ML}$ METHAMPHET AMINE

Table IV. EMIT d.a.u. barbiturate assay results for three case blood samples. Apparent EMIT results with GC results. Two subtherapeutic butalbital spiked blood samples also included.

BARBITURATE ASSAY			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG.CAL	402 404		0.0 $\mu\text{g}/\text{ML}$ SECOBARB
LOW CAL	410		0.10 $\mu\text{g}/\text{ML}$
CASE #1	406 408	NEGATIVE	NEGATIVE
CASE #2	399 400	NEGATIVE	NEGATIVE
CASE #3	403 403	NEGATIVE	NEGATIVE
BUTALBITAL STANDARD	445 447	APPARENT 0.33 $\mu\text{g}/\text{ML}$ SECOBARB	0.50 $\mu\text{g}/\text{ML}$ BUTALBITAL
BUTALBITAL STANDARD	473 474	APPARENT 0.51 $\mu\text{g}/\text{ML}$ SECOBARB	1.00 $\mu\text{g}/\text{ML}$ BUTALBITAL

8. The direct analysis of the same methanolic extract by other methods may be possible in order to confirm an EMIT positive sample or to screen for drugs which are not detected by EMIT. Such techniques might include HPLC, capillary GC with on-column injection and nitrogen specific or electron capture detection, and GC/MS in the SIM mode.

Table V. EMIT d.a.u. benzodiazepine metabolite assay results for four case blood samples. Apparent EMIT results and GC results. One therapeutic triazolam spiked blood sample also included.

BENZODIAZEPINE METABOLITE ASSAY (OXAZEPAM)			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG.CAL	338 340		0 ng/ML OXAZEPAM
LOW CAL	345		150 ng/ML OXAZEPAM
CASE #1	341 339	NEGATIVE	160 ng/ML DIAZEPAM
CASE #2	342 343	NEGATIVE	10 ng/ML DIAZEPAM 20 ng/ML NORDIAZEPAM
CASE #3	538 536	APPARENT >> 600 ng/ML OXAZEPAM	290 ng/ML DIAZEPAM 800 ng/ML NORDIAZEPAM
CASE #4	338 336	NEGATIVE	NEGATIVE
TRIAZOLAM	341 341	NEGATIVE	10 ng/ML TRIAZOLAM

Table VI. EMIT d.a.u. cocaine metabolite (benzoylecgonine) assay results for two case blood samples. Apparent EMIT and GC results.

COCAINE METABOLITE ASSAY (BENZOYLECGONINE)			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG. CAL	461 462		0.0 $\mu\text{g}/\text{ML}$
LOW CAL	467 468		0.15 $\mu\text{g}/\text{ML}$
CASE #1	461 461	NEGATIVE	NEGATIVE
CASE #2	646 652	APPARENT 5.8 $\mu\text{g}/\text{ML}$ BENZOYLECGONINE	4.7 $\mu\text{g}/\text{ML}$ COCAINE (OVERDOSE)

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Table VII. EMIT d.a.u. methaqualone assay results for three case blood samples. Apparent EMIT results and GC results.

METHAQUALONE ASSAY			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG. CAL	275		0.0 $\mu\text{g}/\text{ML}$
LOW CAL	282		0.075 $\mu\text{g}/\text{ML}$
CASE #1	277 279	NEGATIVE	NEGATIVE
CASE #2	278 278	NEGATIVE	NEGATIVE
CASE #3	276 276	NEGATIVE	NEGATIVE

Table VIII. EMIT d.a.u. opiate assay results for three case blood samples. Apparent EMIT results and GC results.

OPIATE ASSAY			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG.CAL	298 299		0.0 $\mu\text{g}/\text{ML}$
LOW CAL	306		0.10 $\mu\text{g}/\text{ML}$
CASE #1	303 302	NEGATIVE	NEGATIVE
CASE #2	320 312	APPARENT 0.13 $\mu\text{g}/\text{ML}$ MORPHINE (STRONG + URINE EMIT ALSO)	0.009 $\mu\text{g}/\text{ML}$ FREE MORPHINE
CASE #3	539 537	APPARENT > 2.5 $\mu\text{g}/\text{ML}$ MORPHINE	0.13 $\mu\text{g}/\text{ML}$ PROPOXYPHENE

Table IX. EMIT d.a.u. phencyclidine assay results for five case blood samples. Apparent EMIT results and GC results.			
PHENCYCLIDINE ASSAY			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG.CAL	298		
LOW CAL	306		
CASE #1	294 296	NEGATIVE	NEGATIVE
CASE #2	296 297	NEGATIVE	NEGATIVE
CASE #3	299 300	NEGATIVE	NEGATIVE
CASE #4	299 291	NEGATIVE	NEGATIVE
CASE #5	299 300	NEGATIVE	NEGATIVE

Table X. EMIT d.a.u. propoxyphene assay results for three case blood samples. Apparent EMIT results and GC results.			
PROPOXYPHENE ASSAY			
SAMPLE	ΔA 340	EMIT RESULTS	GC RESULTS
NEG.CAL	284 285		0.0 $\mu\text{g}/\text{ML}$
LOW CAL	292		0.15 $\mu\text{g}/\text{ML}$
CASE #1	283 287	NEGATIVE	NEGATIVE
CASE #2	282 287	NEGATIVE	NEGATIVE
CASE #3	291 292	APPARENT 0.14 $\mu\text{g}/\text{ML}$ PROPOXYPHENE	0.13 $\mu\text{g}/\text{ML}$ PROPOXYPHENE

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