Int J Leg Med (1992) 105:115–119

International Journal of
Legal Medicine

The screening for common drugs of abuse in whole blood by means of EMIT-ETS and FPIA-ADx urine immunoassays

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Received December 7, 1991 / Received in revised form February 4, 1992

Summary. The purpose of the paper was to compare the performance of ETS (EMIT) and ADx (FPIA) analyzers for screening blood samples for drugs of abuse after 2 alternative pretreatment procedures (acetone precipitation and ultrafiltration). Cannabinoids, benzodiazepines and benzoylecgonine were not detectable with both assays after ultrafiltration. The detectability of morphine in blood ultrafiltrates was distinctly lower than after acetone precipitation. The comparison of results obtained with ETS and ADx after acetone precipitation showed that ETS assay is slightly more sensitive but ADx is slightly more reproducible. Both assays may be used for blood examination with similar cut-off values. The ETS analyzer gave much better analytical performance (speed, flexibility) and lower reagent costs than the ADx analyzer.

Key words: Drugs of abuse – Enzyme multiplied immunoassay (EMIT) – Fluorescence polarization immunoassay (FPIA) – Acetone precipitation – Ultrafiltration

Zusammenfassung. Zum immunologischen Nachweis von Drogen in Blutproben wurde die Brauchbarkeit der ETS (EMIT)- und ADx (FPIA)-Systeme unter Einsatz zweier Probenvorbereitungsmethoden (Ultrafiltration und Acetonfällung) untersucht und die Ergebnisse miteinander verglichen. Cannabinoide (D-8-THC-COOH), Benzodiazepine (Oxazepam) und Benzoylecgonin waren nach Ultrafiltration mit beiden Systemen praktisch nicht nachweisbar. Die Nachweisbarkeit von Morphin war nach Ultrafiltration deutlich schlechter als nach vorausgegangener Acetonfällung. Der Vergleich der mit dem ETS- und ADx-System nach Acetonfällung erhaltenen Ergebnisse zeigt, daß der ETS-Assay etwas empfindlichere, der ADx-Assay besser reproduzierbare Meßergebnisse liefert. Beide Assays sind für Blutuntersuchungen geeignet und lassen vergleichbare cut-off-Werte zu. Das ETS-System zeigt in der Praxis eine bessere Handhabung, Geschwindigkeit und Flexibilität sowie im Vergleich zum ADx deutlich niedrigere Reagenzienkosten.

Schlüsselwörter: Betäubungsmittel – Enzym multiplied immunoassay (EMIT) – Fluorescenzpolarisation-Immunoassay (FPIA) – Acetonfällung – Ultrafiltration

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Introduction

The advent of immunochemical assays in forensic toxicology in the early 1970s changed the whole analytical strategy of screening for drugs of abuse [1–3]. Nowadays, it is taken for granted that toxicologists use the whole spectrum of immunoassays as a screening procedure, and confirm the presumptive positive results with more specific techniques. Among the nonradioactive immunochemical techniques, the enzyme-multiplied immunoassay (EMIT) [4, 5] and fluorescence polarization immunoassay (FPIA) [6, 7] have found broad application in clinical and forensic toxicology, particularly in screening for common drugs of abuse.

When screening large groups of individuals for drugs of abuse, such as pre-employment drug screening or drug abuse prevention programs in industry or military services, a urine sample is the material of choice for analysis [8, 9]. It is therefore understandable, that both companies involved in production of EMIT (Syva) and FPIA (Abbott) assays have developed tests which were primarily targeted for urine. However, in forensic toxicology, particularly in road traffic offence cases, a urine sample is often not available. Moreover, the examination of blood samples gives information which is much more relevant to the actual driving ability. The tentative application of the whole spectrum of immunochemical tests for the screening of blood samples for common drugs of abuse may simplify and accelerate the whole screening procedure.

For these reasons, various authors have adapted existing urine assays for the examination of blood and tissue samples. Slightom et al. [10–12] applied the EMIT assay to blood and tissues subjected to liquid/liquid extraction. Most adaptations are based on the precipitation of blood samples with water-miscible solvents, such as methanol [13–15], acetone [16] or dimethylformamide [17]. All the above-mentioned methods concerned the EMIT assay and demonstrated full applicability of urine assays to blood.

The FPIA urine assay was also adapted to blood without pretreatment [18] after precipitation with acetone [19] or trichloracetic acid [20]. FPIA serum lidocaine, phenobarbital and phenytoin assays, primarily designed for samples from living persons, were successfully used

for examination of hemolytic serum or whole blood [21, 22].

In a previous study we have compared the applicability of the FPIA-TDx and EMIT-dau immunoassays for the detection of 6 groups of common drugs of abuse after acetone precipitation [23]. We found the TDx to be superior to the EMIT-dau AutoCarousel, due to the higher sensitivity for cannabinoids and overall higher robustness. In the meantime, both manufacturers have launched new instrumentation, designed specially for drugs of abuse; ETS Analyzer from Syva and ADx Analyzer from Abott. The ETS Analyzer was found to be superior to the ADx for multi-analytical drug screening with regard to speed, flexibility and sample load [24].

Since both instruments were available to us, we have compared their usefulness for blood screening on drugs of abuse. Two different procedures of sample pretreatment were applied: acetone precipitation [16, 23] and ultrafiltration. The latter technique was applied to opiate screening in blood by means of FPIA-ADx [25], but was not scrutinized as a general sample preparation method for multianalytical screening.

Material and methods

Materials

Five ml samples of autopsy blood, prescreened for the absence of drugs, and 8 samples of blood from a blood bank were spiked with amphetamine, benzoylecgonine, delta-8-THC-carboxylic acid, methadone, morphine, oxazepam and phenobarbital (see Table 1). The spiked samples, as well as blank blood samples, were divided into 2 ml portions and stored at −20°C until analysis.

Sample pretreatment

1. Ultrafiltration. Aliquots of blood (2 ml) were pipetted into Centrisart ultrafiltration cartridges (Sartorius GmbH, Göttingen, Germany), centrifuged at 4000 rpm for 30 min and 200 µl of ultrafiltrate were taken for ADx and ETS determinations.

2. Acetone precipitation. Aliquots of blood (1 ml) were added dropwise to 3 ml acetone, vortexed for 1 min and centrifuged at 5000 rpm for 5 min after which 2.5 ml of clear supernatant was evaporated under nitrogen and reconstituted in 500 µl water for ADx and ETS determinations.

Table 1. Concentrations of drug (ng/ml) in mixed blood standards

Substance	Mixture				
	1	2	3	4	5
D-8-THC-COOH	0	12	25	50	100
Morphine	0	25	50	100	200
Benzoylecgonine	0	25	50	100	200
Amphetamine	0	50	100	200	400
Methadone	0	50	100	200	400
Phenobarbital	0	125	250	500	1000
Oxazepam	0	125	250	500	1000

Immunochemical determinations

FPIA determinations were performed using the ADx Analyzer (Abbott GmbH, Wiesbaden, Germany) and complete ADx reagent kits.

EMIT immunoassays were run on the ETS Analyzer (Syva-Merck AG, Darmstadt, Germany) using Syva-Merck reagent kits.

All immunochemical assays were performed as recommended by the manufacturers' manuals. The measurement values obtained from both methods (netto polarization values for ADx and absorption rate values for ETS) were taken for construction of calibration curves and further analysis. For comparison of sensitivity and precision of both methods, the primary measurement values were recalculated as percent values of those obtained with blank, drugfree blood samples. In the case of ADx assay the net polarization values decreased with increasing concentration of analyte and for better comparison, were changed to positive values.

Results and discussion

Results obtained with ultrafiltrates

Figure 1 shows the comparison of both assays, expressed as percent change of the blank signal. The cannabinoid assay gave negative results in both techniques in all concentration ranges applied. Also, benzoylecgonine and oxazepam were practically undetectable. There are several possible reasons for this finding; the analytes may be adsorbed on the ultrafiltration membrane, or the fraction of non-protein bound substance, which may pass the ultrafiltration membrane, may be too small to be detected by immunoassay. Amphetamine was undetectable in both immunoassays. This finding is in agreement with our previous results with FPIA assay after acetone precipitation of blood [23]. In general it may be stated, that ultrafiltration cannot be used for pretreatment of blood for general drug screening with immunoassays.

Results obtained after acetone precipitation

In this series of experiments the methadone assay was included instead of amphetamine assay. In our previous study we have demonstrated the applicability of FPIA methadone urine assay for blood and tissues after acetone precipitation [26]. Figure 2 shows the comparison of the sensitivity and precision of both tests. It can be stated, that ETS assay is slightly more sensitive. On the other hand, the results obtained with ADx assay were slightly more reproducible. The cut-off values for both tests may therefore be set as follows: Cannabinoids 25 ng/ml, opiates 50 ng/ml, cocaine-metabolite 100 ng/ml, methadone 200 ng/ml, barbiturates 250 ng/ml and benzodiazepines 250 ng/ml. These values correspond to the cut-off values established in other papers [15–18, 20, 23, 26].

The comparison of results obtained with negative calibrators and blank blood samples showed virtually no differences in the net polarization values in ADx assay (Table 2). In the case of ETS some differences between the blank values obtained with urine calibrations and blood samples were observed. Net polarization values of different batches of the same assay, and also of different ADx assays, were of the same order. In ETS assay large differences in delta-absorbance values between different

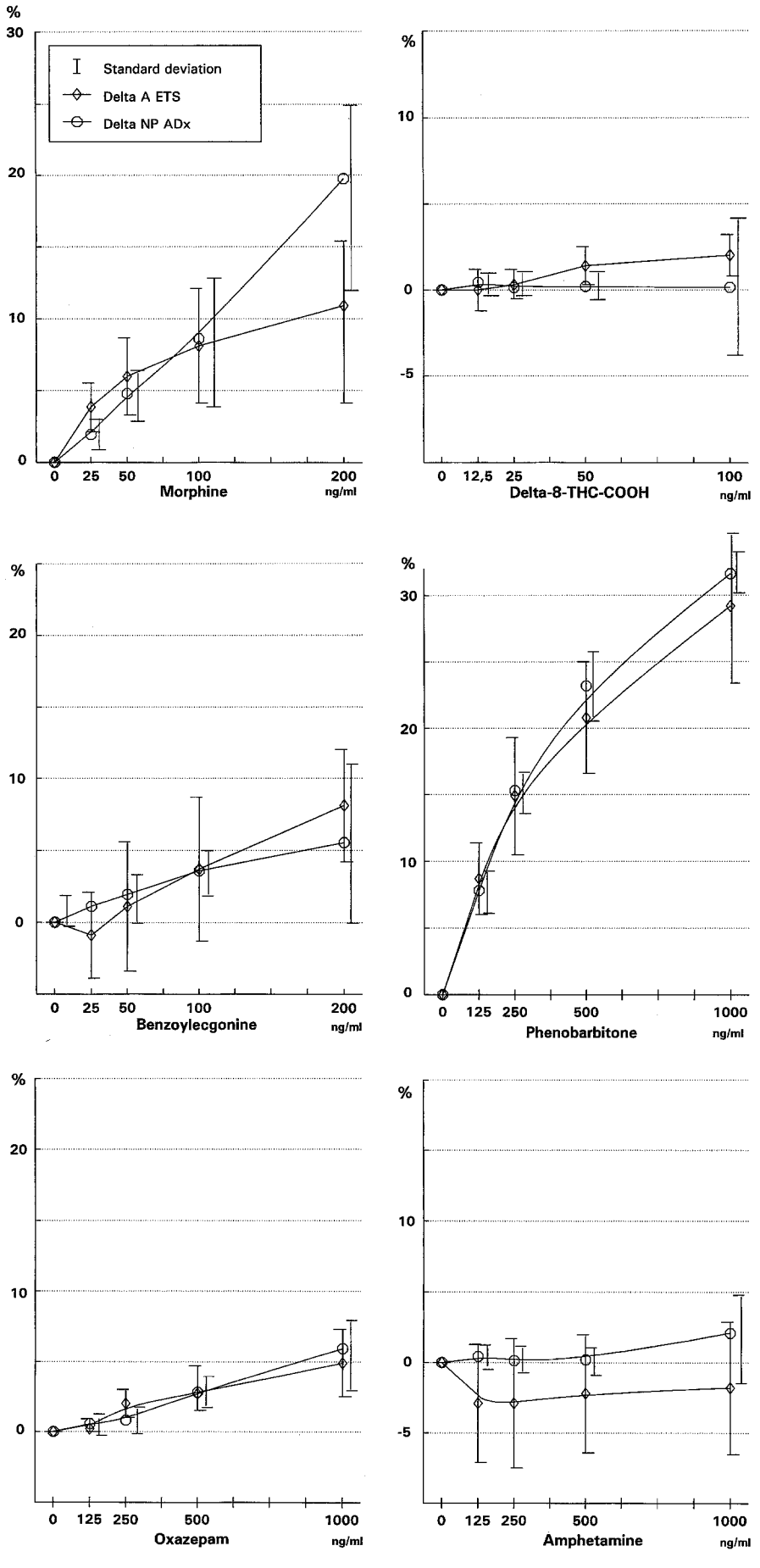

Fig. 1. Sensitivity (expressed as percent change of blank values) and reproducibility (expressed as standard deviation of mean from all experiments) of ETS and ADx assays applied to spiked blood samples subjected to ultrafiltration

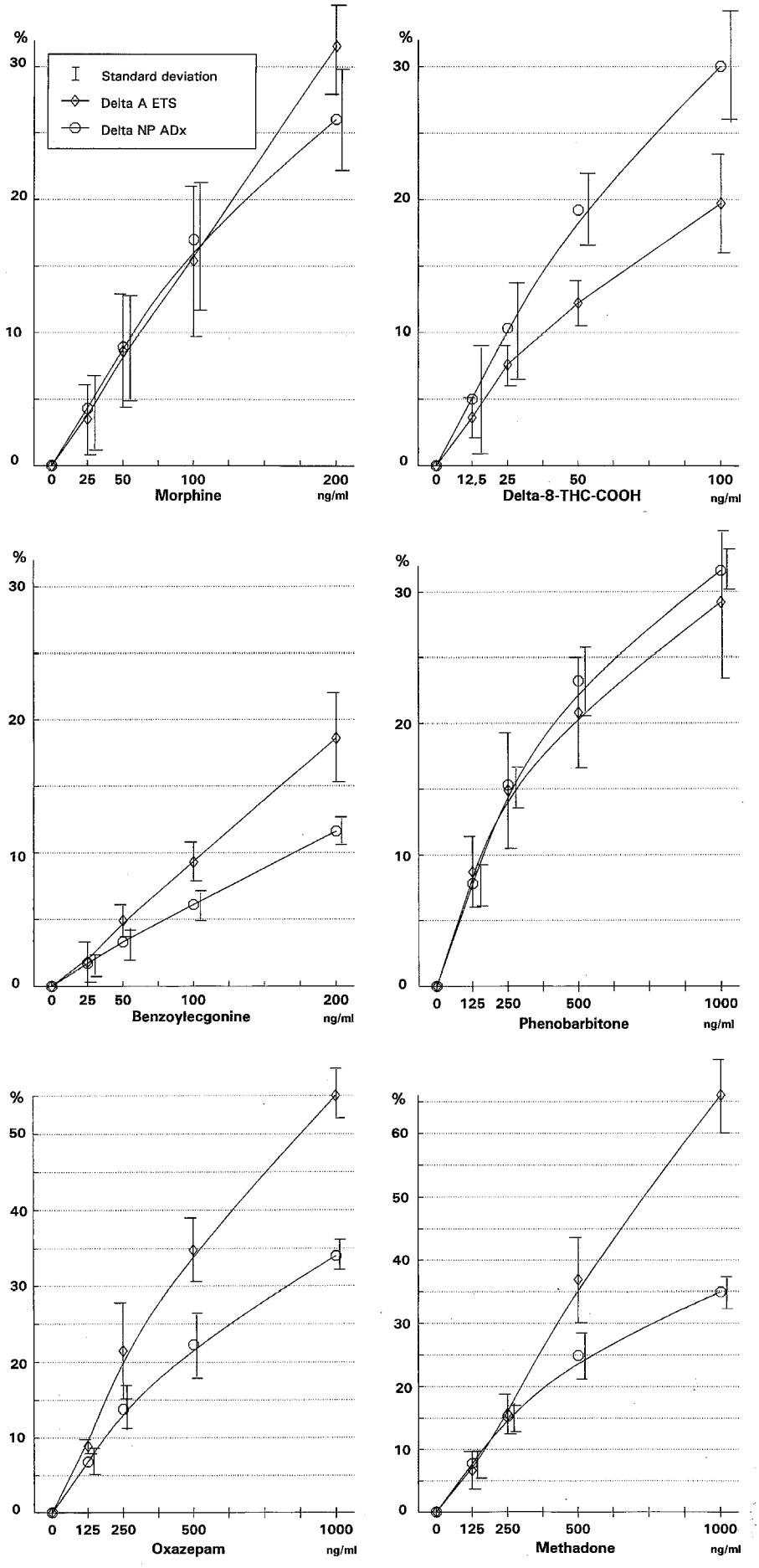

Fig. 2. Sensitivity and reproducibility of ETS and ADx assay applied to spiked blood samples subjected to acetone precipitation

Table 2. Comparison of blank values obtained with negative calibrators and blank blood samples after acetone precipitation for EMIT ETS and FPIA ADx

	ETS (△ A)		ADx (NP)	
	Neg. cal.	Blood	Neg. cal.	Blood
Cannabinoids	845	838 ± 6	165	168 ± 4
Opiates	163	173 ± 4	190	198 ± 2
Cocaine metabolite	272	294 ± 17	195	192 ± 1
Methadone	190	176 ± 19	166	166 ± 1
Barbiturates	488	412 ± 11	167	159 ± 3
Benzodiazepines	463	493 ± 10	211	211 ± 1

batches of the same tests were noted. These findings were in agreement with our observations concerning EMIT-dau and TDx assays [23]. Therefore, blood calibration mixtures containing drugs in cut-off concentrations, must be run with a series of samples analyzed by means of EMIT-ETS. In the case of FPIA-ADx the calibration curves may be prepared for each substance and checked for each new batch of reagents, using calibration drugs at cut-off levels. These procedures are in agreement with the recommendations of both manufacturers for urine assays.

The comparison of analytical performance of both analyzers in routine work showed several advantages of the ETS analyzer. The screening of 12 samples on the 6 groups of drugs examined is twice as fast as the ADx. ETS analyzer prints the results in real time, whereas the results from ADx system are available at the end of the run of all series. If only one sample in the series goes wrong, the whole series is lost. Also, the reagent costs are more than twice as high in ADx assay, despite high consumption of reagents for calibration in ETS.

Conclusions

1. Acetone precipitation is fully applicable as a blood pretreatment for screening of common drugs of abuse with EMIT-ETS or FPIA-ADx immunoassays. Ultrafiltration of blood samples may be applied only for opiates and barbiturates and cannot be used for general screening procedure.

2. Both analyzers – ETS and ADx – may be used for blood screening with similar efficiency. ETS analyzer is superior due to a much better analytical performance in everyday use and lower reagent costs.

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