Journal of Chromatography, 245 (1982) 129–132
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,982

Note

Comparison of methods of analysis for amphetamine and related drugs

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(Received April 22nd, 1982)

Amphetamine drugs (amphetamine, methamphetamine, phentermine, ephedrine, phenylpropanolamine, phenmetrazine, and phendimetrazine) are frequently used and abused. Forensic laboratories, hospital emergency room laboratories, and toxicology laboratories are frequently requested to analyze for their presence. Procedures utilizing gas chromatography (GC)[1–6], enzyme multiplied immunoassay technique (EMIT)[6–8], and radioimmunoassay (RIA)[9,10] have been developed for the analysis of amphetamine and related drugs. This study will attempt to show systematically the advantages and disadvantages of these three classes of methods so that laboratories can more easily choose the best method for their needs.

MATERIALS AND METHODS

The EMIT analyses[7] were performed using equipment and reagents available from Syva Corp. (Palo Alto, CA, U.S.A.). The EMIT analyses are performed as follows. To a plastic beaker add 0.2 ml of bacteria suspension, 50 μl of sample followed by 250 μl of buffer solution, and 50 μl of amphetamine antibody reagent followed by 250 μl of buffer solution. When ready for analysis, add 50 μl of enzyme reagent followed by 250 μl of buffer solution to the beaker, then immediately aspirate the contents of the beaker into the spectrophotometer flow-cell. The optical density will be measured at 10 sec and at 50 sec after aspiration into the flow cell. The difference (ΔOD) will be printed out and can be compared with that of the standards.

The RIA analyses were performed using automatic pipettors available from Micromedic, a γ scintillation counter available from Packard, and reagents available from Roche Diagnostics (Nutley, NJ, U.S.A.). The RIA analyses are done as follows. Three sets of tubes are set up for each concentration of each sample or standard to be analyzed. Add about 1 ml of each sample to the appropriate tube. Using an automatic pipetting station, add 0.1 ml of each solution to be analyzed from the above tubes, 0.2 ml of ^{125}I-amphetamine antigen, and 0.2 ml of amphetamine antibody to a set of tubes. Incubate the reaction mixture at room temperature for 1 h. After incubation, add 0.5 ml of saturated ammonium sulfate to precipitate the protein complexes. Allow the reaction mixture to incubate at room temperature for 10 min. After incubation, centrifuge the tubes at 2000 *g* for 10 min. After centrifugation use an auto-

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0021-9673/82/0000–0000/$02.75

matic pipetting station to withdraw 0.5 ml of the supernatant fluid and add that supernatant fluid followed by 0.5 ml of distilled water to another tube for each tube of the reaction mixture. Count the tubes of supernatant fluid for 1 min in a gamma scintillation counter. Compare the sample counts with those of the standards.

The GC analyses were performed using equipment available from Varian Assoc. (Palo Alto, CA, U.S.A.). The GC analysis is performed as follows. To a culture tube add 7 ml of urine, 1 ml of 1 *N* sodium hydroxide solution and 7 ml of extraction solvent (isopropanol–chloroform containing 1 μg/ml *n*-propylamphetamine). Shake the tubes at slow speed on a platform shaker for 15 min, then aspirate and discard the top (urine) layer. Add 1 ml of 0.2 *N* sulfuric acid, then shake the tube at high speed for 5 min. Transfer the acid layer to a 5 ml centrifuge tube containing 0.2 ml of 1.5 *N* sodium hydroxide solution and 0.1 ml of chloroform. Vortex the mixture for 1 min then centrifuge the tube for 5 min. Inject 2–3 μl of the chloroform extract into the gas chromatograph containing a 6 ft. × 2 mm I.D. glass column packed with 10% Apiezon L–10% KOH on Chromosorb W NAW, 80–100 mesh, operated at 150°C.

RESULTS AND DISCUSSION

Tables I and II present a thorough comparison of the three methods —RIA[9], EMIT[7], and GC using an Apiezon–KOH column[6]. Table I presents objective com-

TABLE I

OBJECTIVE COMPARISON OF METHODS

	GC	*EMIT**	*RIA***
Minimum concentration detectable (μg/ml)			
Amphetamine	0.4	1.0	0.1
Methamphetamine	0.4	0.7	5.0
Phentermine	0.4	2.5	ND
Ephedrine	2.0	7.0	ND
Phenylpropanolamine	2.0	7.0	ND
Phenmetrazine	1.5	3.0	ND
Phendimetrazine	1.5	ND	ND
Differentiation amphetamine drugs	Yes	No	No
Interference from β-phenethylamine	Little	Yes	None
False positives	Very rare	Very rare	Occasional
False negatives	None	None	None
Equipment cost	Moderate	High	High
Reagent cost	Low	High	High
Quantitation	Excellent	Fair	Fair
Objectivity in results interpretation	Yes	Yes	Yes
Adaptability for mass screening	Some	Excellent	Excellent
Length of analysis/1000 samples	Slow	Moderate	Moderate fast
Length of analysis/sample	Moderately fast	Very fast	Slow
Biological samples	Biological fluids	Urine	Biological fluids

* Values from laboratory testing; slightly lower than those reported by Syva.

** Values from laboratory testing.

TABLE II

METHOD COMPARISON

	GC	*EMIT*	*RIA*
Advantages	1 Detects and differentiates most amphetamine drugs	1 Detects many amphetamine drugs	1 Detects amphetamine only
	2 Best for quantitation	2 No false negatives	2 Best for many samples
	3 Good sensitivity	3 Fastest for a single sample	3 All biological fluids can be analyzed
	4 No false negatives or positives*	4 Good for many samples	4 Best amphetamine sensitivity
	5 Low reagent cost		5 No false positives
	6 Least equipment cost		6 No interference from β-penethylamine in decomposed samples
	7 Fairly fast for a single sample		
	8 Little interference from β-phenethylamine in decomposed samples		
	9 All biological fluids can be analyzed		
Disadvantages	1 Not readily adapted for mass screening unless low positive rate	1 Only urine can be used	1 No other common amphetamine drugs detected
	2 Slowest for many samples	2 High equipment cost	2 Very slow for single samples
		3 High reagent cost	3 High equipment cost
		4 Interference from β-phenethylamine in decomposed samples	4 High reagent cost
		5 Worst sensitivity	
		6 Occasional false positives	
		7 No differentiation between amphetamine drugs	

* If derivative confirmation done[5].

parisons of the methods' features. Table II compares the advantages and disadvantages (strengths and weaknesses) of each.

Table II illustrates that each method has inherent advantages and disadvantages. Thus, in making a decision as to which method is best, several very important factors must be considered. First, the type of biological sample to be tested must be known. All three methods can be used for urine, but only GC and RIA can be used for other biological fluids such as blood. (Note that while the extraction procedure described is designed for urine, other procedures[1,2] have been developed for extracting amphetamine from other tissues.) Second, it must be decided which amphetamine drugs are to be detected. RIA essentially detects only amphetamine itself[10], while both EMIT and GC can detect other amphetamine drugs[6,8]. Gas chromatography, of

course, has the widest range of detection and the best sensitivity. Third, it must be decided if the ability to differentiate amphetamine drugs is necessary. Only GC can both detect and differentiate other amphetamine drugs. When these three factors have been evaluated for a laboratory situation, one will probably be left with only one method (two at most) that will fully meet the needs.

RIA is best suited for large-scale analysis programs (more than 50 samples at a time) where only amphetamine of the various amphetamine drugs is to be analyzed for and where any type of biological fluids might be submitted for analysis.

GC is best suited for small-scale analysis programs (1–20 samples at one time) where any of the common amphetamine drugs are to be analyzed for, differentiated, and accurately quantitated; where any type of biological fluid might be submitted for analysis and where results for one sample could be needed within 30 min.

EMIT is best suited for both small-scale and large-scale analysis programs where fresh urine is the specimen submitted for analysis and where a result of amphetamine-drug present or absent without differentiation or accurate quantitation is all that is needed.

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